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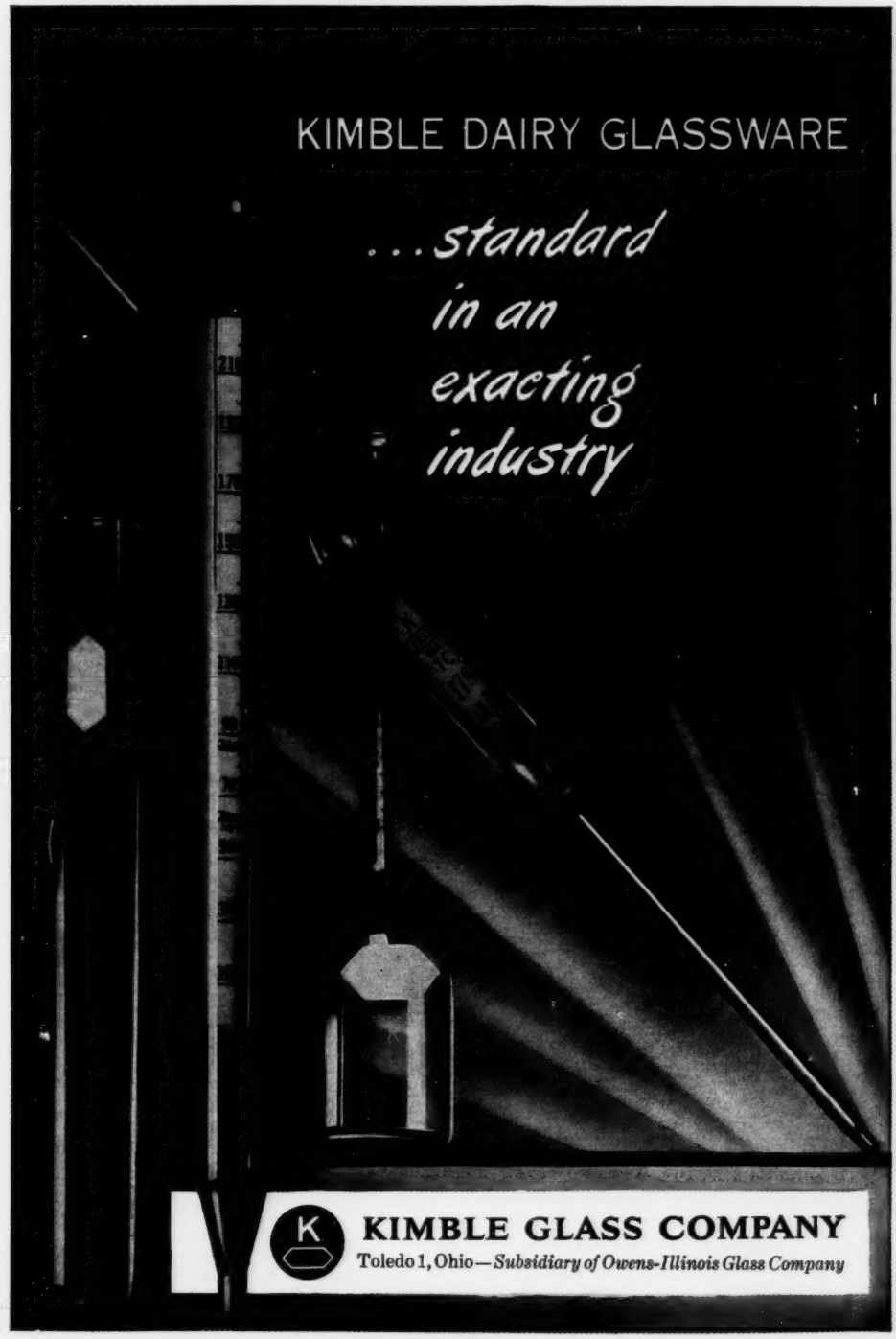

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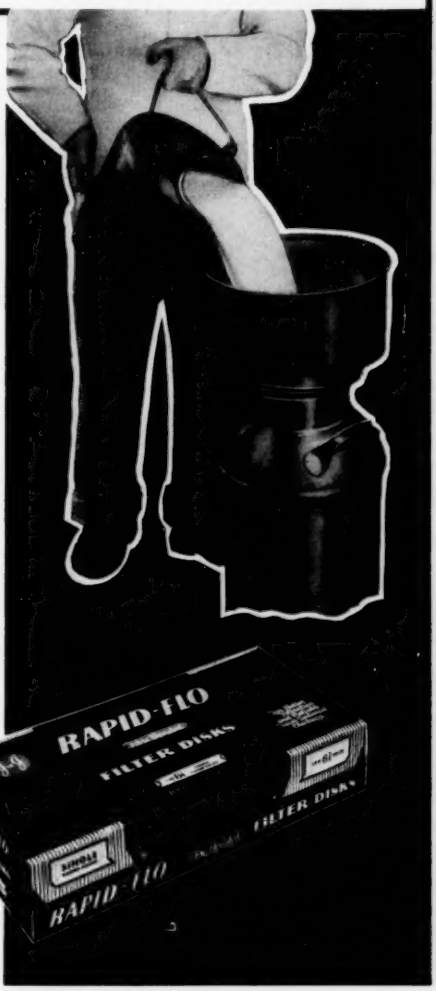

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THE EFFECT OF LIGHT ON ACTIVATED FLAVOR DEVELOPMENT AND ON THE CONSTITUENTS OF MILK AND ITS PRODUCTS: A REVIEW[1]

J. W. STULL
Department of Dairy Husbandry
University of Arizona, Tucson

INTRODUCTION

An effect of light on the flavor of milk products was reported in Europe as early as 1890 by Hanus (37) and later, in 1907, by Burr (11). These investigators did not elucidate the exact nature of the flavor defect observed. In the United States, references to the subject were made by Hammer and Cordes (36) in 1920 and by Frazier (31) in 1928.

In milk, the problem of light-induced off-flavor development was brought into sharp focus during the late 1920's and early 1930's with the advent of vitamin D fortification by ultraviolet irradiation. Drummond (25), however, sounded an ominous note in 1927 when he conjectured that the benefits of irradiating milk might be outweighed by the possibilities of off-flavor development and destruction of photo-sensitive vitamins.

In all the early reports, the character of the flavor developed in milk on exposure to light was apparently assumed to be singular in nature. In 1931, however, Davies (18), Tracy (77), and Tracy and Ruehe (83) pointed out that there were, in fact, two distinct flavors that might be observed in milk due to the action of light energy. The first of these flavors was the then familiar oxidized or tallowy defect. The literature dealing with this off-flavor has been reviewed by Brown and Thurston (8) in 1940 and by Greenbank (33) in 1948.

The second and distinctly different defect was described by these authors (18, 77, 83) as "sunlight" or "burnt." Weckel and Jackson (87, 88, 89) later suggested that the flavor be referred to as the "activated" flavor. Other terms such as "burnt feather," "burnt protein," "scorched," "cabbage," and "mushroom" have been used to characterize the flavor defect.

Even though the conception of the dual nature of oxidative flavor changes in milk had been generally recognized since 1931, investigators in Australia (2) reported in 1950 that the activated flavor was a separate problem in their country.

Of further interest has been the announcement by Weinstein and Trout (92) of an atypical flavor development in milk exposed to daylight. They described

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[1] Arizona Agricultural Experiment Station Technical Paper 328.

it as "unclean," "nauseating," or "of decomposed protein, suggesting that of milk noted from cows having a serious physiological disturbance."

The light induced flavor defect to be considered in this review is the activated flavor.

FACTORS AFFECTING DEVELOPMENT OF ACTIVATED FLAVOR IN MILK

Homogenization. Realization of the problem of activated flavor development was brought about by two factors; *(a)* the advent of vitamin D fortification by ultraviolet irradiation, and *(b)*, perhaps the more important factor, the great increase in the application of the homogenization process to market milk supplies, commencing about 1930 (84). Hood and White (44) in 1934 were the first to emphasize that homogenization increased the susceptibility of milk to activated flavor development. This fact has since been supported by many investigators (3, 10, 15, 16, 17, 20, 21, 22, 23, 24, 29, 38, 79, 80, 81).

Flake *et al.* (26) stated that in the production of vitamin D fortified milk, irradiation prior to homogenization resulted in less trouble from activated flavor development. Weinstein and Trout (93) found, however, that homogenization after exposure to solar radiation accelerated the development of the flavor. Tracy (78) believed that the effect of light was more noticeable in homogenized milk because of the increased area of protein surface adsorbed on the fat globule.

Heat treatment, aging. Stamberg and Theophilus (69) observed that raw milk was more susceptible to activated flavor development than was either pasteurized milk or homogenized milk. Weinstein and Trout (93), on the other hand, concluded from their work that heating milk to 175° F. for 5 minutes did not retard the development of the flavor. These workers found that solar radiation actually tends to destroy the cooked flavor imparted to milk by the high heat treatment. Josephson (48) reported that freshly pasteurized milk was more susceptible than the same milk after aging.

Light source. Several methods of supplying light energy have been reported in studies dealing with the effect of light on the development of activated flavor. The most common method was to expose the sample to sunlight. In these cases, the duration of exposure, time of day, season, geographical location, and presence of clouds (if any) were specified in whole or in part.

Holmes and Jones (43) recorded the intensity of the sunlight by the use of a pyrheliometer. Another method involved supplying light energy from mercury vapor lamps and various types of arc sources. In these cases, assuming that the performance of the electronic sources was controlled, the intensity of the light energy could be standardized.

Francis (30) made a study of the effect of various types of fluorescent lights on the development of the activated flavor. The amount of light energy absorbed was controlled by distance and rated output of the lights. This investigator found that in the order of greatest effect on the flavor of milk were the fluorescent tubes described as producing "white," "daylight," and "soft white" light, respectively.

Length of exposure to light. Depending on the amount of light energy

absorbed by the sample, activated flavor has been reported to be discernible in as short an exposure time as 10 minutes (41). Of striking interest in this connection is the fact that Josephson (48) in 1946 and Herreid *et al.* (41) in 1952 observed that on prolonged exposure (e.g., 1 to 2 hours) the intensity of the activated flavor decreases and may totally disappear. This phenomenon apparently is due to the oxidation of the flavor component to a flavorless compound (s).

Additional support for this explanation was the earlier report in 1938 by Flake, Weckel, and Jackson (26) that oxidation might destroy the flavor. These authors reported that the oxidative action of hydrogen peroxide, oxygen, and cupric ions would remove the activated flavor.

Wave length of light. It was recognized during the early 1930's that ultraviolet irradiation of milk might produce a flavor defect. This indicated that short wave lengths of light were at least partly responsible for the development of what was later called the activated flavor. By the use of colored glass filters, Flake *et al.* (29) studied the effect of various wave lengths of light on flavor development. They concluded that filters which eliminated all wave lengths less than 460 mμ were very effective in preventing development of activated flavor in milk exposed to sunlight. Using the same technique, Josephson (48), reported that to prevent the development of activated flavor in exposed milk, a filter which eliminated all wave lengths below 620 mμ was required. These results have been confirmed by Herreid *et al.* (41) who found that ruby glass bottles gave almost complete flavor protection to milk exposed to sunlight. The light transmittance curve given for this container showed that only a small amount of light was transmitted below 620 mμ and none below 484 mμ.

Temperature. The literature reports the temperature of the milk and atmosphere during exposure to light in only a few cases. For example, Francis (30) exposed samples to artificial light in a room maintained at 42-44° F. Holmes and Jones (43) recorded the temperature of the milk after exposure to sunlight.

Container. In 1907, Burr (11) suggested the use of red, green, or black bottles as containers for milk. The study of Hammer and Cordes (36) in 1920 proved the value of using colored (brown) glass in preventing the action of light on the flavor of milk. The application of this principle was not accepted at that time since the consumer was "cream-line conscious." With the widespread introduction of homogenized milk and the single service container, this consumer reaction has been largely allayed.

Several authors (30, 41, 68, 101) have observed that containers made of amber glass or paper offer actinic protection, whereas containers made of clear (flint) glass offer little or no protection against the effect of light on development of activated flavor. Amber glass offered almost complete protection from sunlight exposure up to 30 minutes (41) and in artificial light (30) up to 4 hours. Herreid *et al.* (41) included in their work a study of a ruby glass container and found that it possessed the greatest actinic protection of all containers. Henderson *et al.* (39) conducted a study of bleached and unbleached paper containers.

The unbleached paper containers gave almost complete protection against flavor change upon exposure to sunlight.

Salt balance. Flake *et al.* (26, 29) have shown that altering the salt balance by the addition of sodium citrate, sodium phosphate, calcium acetate, calcium chloride, calcium lactate, or calcium phosphate did not change the susceptibility of milk to activated flavor development.

Antioxidants. Since oxidation causes activated flavor, it has been observed that compounds with antioxygenic properties retard its development. Swanson and Weckel (73) and Flake *et al.* (29) found that oat flour derivatives (Avenex) retarded activated flavor development. Weinstein and Trout (93) reported results using several compounds as antioxidants and synergists. They found that the addition of ascorbic acid accelerated the oxidation of the milk constituent responsible for the activated flavors. These authors also studied nordihydroguaiaretic acid (NDGA), alpha-tocopherol, and hydroquinone alone and in combination with ascorbic acid. They showed that the addition of 25 mg. per liter of NDGA alone or 75 mg. per liter in combination with ascorbic acid prevented activated flavor development. Alpha-tocopherol and hydroquinone added separately or in combination with ascorbic acid did not give complete protection against the flavor development.

Exclusion of air. The oxidative nature of activated flavor development indicates that a reduction of oxygen tension in milk would retard the occurrence of the flavor. Swanson and Weckel (74) and Flake *et al.* (29) replaced the oxygen in milk with nitrogen and observed that the flavor did not appear. Weinstein and Trout (94) deaerated milk under 29 in. of vacuum and stated that no activated flavor developed after exposure to sunlight for 1 hour.

Breed, fat content, feed, individuality, stage of lactation. Weinstein and Trout (92) studied these factors and found that there seemed to be no correlation between breed, fat content of milk, or stage of lactation and the susceptibility of milk to activated flavor development. These investigators reported that individual samples of milk from about 30% of cows receiving green feed failed to develop activated flavor after 30-60 minutes exposure to solar radiation. Milk from all cows fed dry feed was susceptible to the flavor defect. This showed that there was variation in the susceptibility of milk from individual cows. It also indicated that milk produced on green feed was less susceptible to photocatalytic flavor change. This latter point has been confirmed by Herreid *et al.* (41).

ACTIVATED FLAVOR IN MILK PRODUCTS

Butter. The light-induced off-flavor first reported in butter by Hanus (37) in 1890 and later by others (34, 46, 52, 75, 98, 99) was undoubtedly a tallowy or oxidized defect rather than a strictly activated flavor. Extensive reviews of the literature on this subject have been made (8, 33).

Buttermilk. Doan and Meyers (24) found that both "plain" buttermilk with butter granules and "creamed" buttermilk developed a very obnoxious odor

like sauerkraut on exposure to sunlight. No tallowy flavor was observed in the samples.

Cottage cheese. Tracy and Ramsey (82) described an off-flavor in cottage cheese due to the effect of exposure to light. This flavor was apparently different from the typical tallowy defect.

EFFECT OF LIGHT ON VITAMIN DESTRUCTION

Vitamin A and carotene. Early workers were concerned about the effect of irradiation on possible vitamin destruction. In the period 1927 to 1933, therefore, a number of investigators (4, 5, 13, 32, 61, 62, 63, 64, 73) reported that milk irradiated to produce from 135 to 400 U.S.P. units of vitamin D per quart showed little or no vitamin A or carotene loss. On the other hand, prolonged exposure has been shown to be deleterious to the vitamin A and carotene content of milk (19, 25, 76, 85), butter (67, 104), milk fat (54, 55, 73) and nonfat milk solids (47).

Vitamin B_1 (thiamin). Cannon and Hixon (13), Fuhr *et al.* (32), Schoenleber and Steenbock (62), and Rohr and Schultz (61) claimed that ultraviolet irradiation did not destroy vitamin B_1, whereas De (19) pointed out that prolonged exposure will reduce the content of this vitamin.

Vitamin B_2 (lactoflavin, riboflavin). Burgwald and Josephson (9) found that periodic exposure of milk to room daylight (1-2 hours a day for as long as 12 days) did not affect the riboflavin content. Likewise, Fuhr *et al.* (32) stated that milk irradiated to produce 400 U.S.P. units of vitamin D per quart was not reduced in riboflavin content.

In sunlight, however, loss of riboflavin is rapid (41, 42, 49, 58, 65, 69). In 30 minutes up to 30% loss has been recorded and in 120 minutes up to 80% destruction was reported (41). Prolonged exposure to artificial light sources also will cause destruction of riboflavin (35, 48, 84, 100, 101, 103).

Vitamin B_6 (pyridoxin). György (35) found that the pyridoxin content of milk could be reduced by exposure to artificial light.

Vitamin C (ascorbic acid). Ascorbic acid is readily oxidized to the dehydro form. The speed of this reaction is greatly accelerated in the presence of light. Burgwald and Josephson (9) found that 1-2 hours exposure a day to room daylight caused complete disappearance of ascorbic acid in four days. Josephson *et al.* (49) reported that exposure of milk in containers ranging in size from ½-pt. to 2-qt. for 1 to 2 hours in shade resulted in 30 to 80% destruction of vitamin C. In sunlight, there may be 100% oxidation of ascorbic acid in 10 to 30 minutes in clear glass bottles (12, 41, 43, 49, 53). Containers made of paper or green, amber (brown) or ruby glass offer effective protection against the destruction of vitamin C in milk exposed to sunlight (41, 43, 45). Kon and Watson (53) reported that replacing the oxygen in milk by an inert gas prevented loss of ascorbic acid during 2 hours exposure to sunlight.

Vitamin D. Although ultraviolet irradiation of milk will produce vitamin D from its precursor, ergosterol, Steenbock and Wirick (72) reported that excessive irradiation would destroy the antirachitic potency of milk.

Vitamin E. Krukovsky *et al.* (56) concluded from their studies that exposure of pure milk fat to artificial light (mercury vapor lamp) slightly reduced the vitamin E content of the fat.

CHEMICAL CHANGES IN MILK CONSTITUENTS DURING ACTIVATED FLAVOR DEVELOPMENT

Milk fat (butterfat). The terms *milk fat* and *butterfat* have been used somewhat synonymously. Both terms have reference to a complex aggregation of milk constituents which include the true fats or glycerides, naturally occurring free fatty acids, phospholipids, and other associated fat-soluble compounds, such as beta-carotene, vitamin A, tocopherol, cholesterol, and ergosterol. The action of light on milk fat is to hasten the oxidation of one or more of the components of this material (40, 66, 70). These reactions give rise to the development of the oxidized flavor (8, 33). Since this type of flavor change can occur prior to, concurrent with, or subsequent to, the activated flavor development, early investigators did not completely resolve the complex nature of the oxidation of milk constituents (18, 77, 83).

Vitamins. The photolytic destruction of riboflavin was thought to have a direct bearing on activated flavor development after Doan and Meyers (24) in 1936 concluded that the appearance of the flavor coincided approximately with the fading or destruction of riboflavin (lactoflavin). In 1940, however, Flake *et al.* (27) advanced the proof that riboflavin and other dialysable substances would not cause activated flavor on exposure to light. Recently, Weinstein and Trout (92) found that adding riboflavin and ascorbic acid to hydrogen peroxide-treated milk intensified the activated flavor. These authors explain their observation in terms of Reed's (59) report that the oxidation of ascorbic acid (a reducing compound) is accelerated in the presence of riboflavin. This loss of reduction potential then would accelerate the development of activated flavor.

Henderson *et al.* (39) were confident that the effect of sunlight on ascorbic acid destruction could be used as a useful index for the effect of light on activated flavor development.

Krukovsky *et al.* (55) observed that the destruction of vitamin A by ultraviolet light was not dependent on the availability of atmospheric oxygen. The destruction of carotene, on the other hand, was dependent on the presence of oxygen and was accompanied with considerable accumulation of peroxides. These authors concluded that the photolytic destruction of vitamin A was a unimolecular reaction but that the disappearance of carotene involved the oxidation of double bonds. Zilva (104) found that irradiating milk fat in carbon dioxide prevented the destruction of vitamin A.

Protein. By 1933, workers in other fields (6, 7, 14, 60, 71, 97) had shown that strong sources of ultraviolet light energy would cause certain changes in protein. These changes included coagulation, liberation of SH groups, and production of peculiar odors and flavors. It was pointed out by Anderson and Tribold (1) that irradiating milk did not affect the amount of casein or albumin.

Weckel (86) found, however, that upon absorption of radiant energy, milk exhibited a delay in the time required for rennet coagulation. Tracy (78) in 1935 was the first to consider that the development of activated flavor was probably an effect of light on milk protein. Next it was pointed out in 1936 by Doan and Meyers (24) that the flavor originated in the casein and albumin-free serum of milk. This conclusion was confirmed by Keeney and Josephson (51).

In 1940, Flake *et al.* (27, 28), described a series of experiments which indicated that a sulfur-bearing compound was the source of activated flavor. Irradiating amino acid systems led these authors to believe that the sulfur-bearing compounds, cystine and methionine, as well as tryptophane and histidine, were important contributors to the flavor. Keeney and Josephson (51) reached the same general conclusions in 1947. Patton and Josephson (57) have reported recently that methionine is the principal source of activated flavor and that flavor production depends, in a large measure, on the presence of riboflavin.

Josephson (48) pointed out in 1946 that the development of activated flavor was an oxidation-reduction reaction. His conclusion was that the reaction probably resulted in the formation of complex reducing substances which actually had an antioxygenic effect on oxidizable milk lipids.

In 1949, Keeney (50) showed that the diffusate from dialyzed skimmilk would not give rise to activated flavor. At the same time, however, dialysis of skimmilk caused a decrease in flavor development and adding the diffusate back to the dialyzed milk increased the flavor production. Keeney (50) isolated a crude preparation of the flavor component and showed that it was heat flocculable and basic in reaction.

Finally, in 1951, Weinstein *et al.* (90, 91, 95, 96) isolated a minor whey protein fraction which gave rise to the flavor. This fraction was not identical with any of the characterized whey proteins. It had a minimum molecular weight of 70,300. Electrophoretic studies showed that the fraction was composed of at least two components or complexes.

SUMMARY

Light from either natural or artificial sources causes chemical change(s) in a minor whey protein constituent of milk. These changes give rise to the development of an off-flavor commonly described as the activated flavor.

Homogenized milk is more susceptible to this defect than is unhomogenized milk. It is believed that the increased area of protein surface adsorbed on the fat globules accounts for this greater susceptibility.

The activated flavor may be detected in milk in clear glass bottles after exposure to sunlight for as little as 10 minutes. Prolonged exposure (1 to 2 hours) tends to decrease the intensity of the flavor. This phenomenon apparently is due to the further oxidation of the flavor compounds to flavorless components.

The development of activated flavor may be retarded by the exclusion of air from milk. The flavor may be prevented by the addition of combinations of synergists and antioxidants, such as ascorbic acid and nordihydroguaiaretic acid. Containers made of ruby or amber glass or paper offer actinic protection to milk.

There is no correlation between heat treatment, salt balance, breed, fat content, or stage of lactation and the susceptibility of milk to activated flavor development. The flavor develops more readily in milk produced by cows on dry feed than in that of cows on green feed. Milk from some cows seems to be more susceptible to activated flavor development than is the milk from other cows.

Irradiation of milk to produce 135 to 400 U.S.P. units of vitamin D per quart does not cause appreciable loss of photosensitive vitamins. Prolonged exposure to natural or artificial light, however, may cause destruction of carotene, vitamins A and D, as well as thiamin, riboflavin, pyridoxine, ascorbic acid, and tocopherol in milk and its products.

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THE INFLUENCE OF TESTES BIOPSY ON SEMEN QUALITY[1]

JOHN H. BYERS
Department of Dairy Husbandry
Oregon State College, Corvallis

Vitamin A and carotene are known to influence the reproductive performance of bulls. The effects of various levels of vitamin A or carotene, or both, on the development of the reproductive system, particularly during the first year of the animal's life, have not been thoroughly explored.

When working with dairy bulls, the excessive cost of maintenance necessitates limiting the numbers of experimental animals, and this in turn demands gathering as much information on each individual as time and techniques allow. It is agreed that the standard methods of evaluating semen quality are not entirely adequate. Since vitamin A affects semen quality, and in particular the rate of spermatogenesis, information on the histological makeup of the testes and a correlation of this with semen characteristics would be of value. Prior to the development of the biopsy technique, no information on the development of the testicle was available unless an autopsy was performed or one or both testicles were completely removed. Such technique is obviously very limiting.

With the development of the biopsy technique, it is now possible to get additional information on a single animal, for by removal of small pieces of tissue many studies of varied treatments may be made.

Erb *et al.* (2) report the use of the biopsy technique in studying the effects of vitamin A deficiency and therapy upon spermatogenesis and semen quality.

Andrews and McKenzie (1) report doing 28 biopsies on the genital system of a single mare within a 3-month period. Sykes *et al.* (3) in studying the effect of restricted energy intake on semen quality with the biopsy technique found changes in semen characteristics and in testes morphology that were unrelated to dietary conditions.

In view of doubts cast on a technique that it was hoped would allow study of testicular development under varying vitamin A and carotene levels, a study was undertaken to determine the effects, if any, of the biopsy technique on semen characteristics of bulls raised under conditions and on rations considered to be normal.

MATERIALS AND METHODS

Studies were made on ten bulls of the Jersey and Holstein breeds ranging in age from 8 to 36 months. All ten bulls were from dams which had been carried on a ration of pasture in season, alfalfa hay, grass silage, and a complete grain mix throughout their pregnancy. The bulls have been raised on a normal ration.

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The biopsy technique differed from that of Erb *et al.* (2) and that of Sykes *et al.* (3). The bull was thrown and securely tied with ropes, but no anesthesia was used. The hair was shaved from the lower scrotal sack with a safety razor and the area thoroughly cleansed with 70% alcohol. The bottom of the scrotum was drawn up tightly by one of the two operators over that point of the testicle having the greatest diameter. An incision approximately ½ inch long was made in the scrotum and through the tunica albuginia. This step in the technique allowed for good drainage, for when the operation was completed the part of the scrotum with the incision settled at the lowest possible position. The scalpel was thrust at a right angle to and into the center of the testicle. The scalpel then was removed and a curved biopsy punch (designed for humans) was inserted in the incision. Pieces of tissue measuring ¼ inch in diameter were removed from each side of the incision. One each of the specimens was placed in Bouin's and Zenker's fixative. Staining with Harris' Hematoxylin was standard procedure. Aseptic precautions were practiced at all times. No attempt was made to stop bleeding and no suturing was practiced.

Two Holstein bulls, each 14 months of age, were biopsied in each testicle simultaneously. A second biopsy of the same testicle 1 year later was performed on one Holstein and one Jersey bull.

All semen collections were made 2 days after the biopsy. Semen evaluation was made on the fresh, undiluted semen and on diluted semen stored at 4° C. When possible, cows were inseminated with semen collected both before and after the biopsy, or data from natural service were accumulated when available.

Six months after the biopsy operation, Jersey Bull 215B1 and Holstein bull 456B2 were slaughtered, and histological examination was made of the testicles. Bull 359B1 was slaughtered 15 months after the simultaneous biopsy of right and left testicles.

RESULTS

Table 1 shows the pertinent data with respect to each bull before and after the biopsy, and for bulls 456B1 and 210B2 before and after the second biopsy of the same testicle.

The quality of semen improved except for one Jersey and two Holstein bulls. With bulls 456B1 and 210B2, the second biopsy lowered the total spermatozoa and slightly increased the per cent abnormal spermatozoa. In bull 456B1, the per cent motile spermatozoa was lowered somewhat after the second biopsy. It had not been possible to get a collection from bull 210B2 before the first biopsy, but immediately after biopsy a good sample of semen was obtained. In bull 456B2 complete azoospermia developed after biopsy of the right and left testicles simultaneously.

There was not opportunity to breed the bulls to many cows, but where breeding data were obtained, the results were satisfactory.

Table 1 shows that although the number of cows bred to each bull is small, the efficiency of breeding by the respective bulls has not been influenced ap-

TABLE 1

Average volume, per cent abnormal, concentration, per cent motility and breeding efficiency of semen before and after biopsy

Bull	Age	No. of observations	Volume	Concentration	Motile	Abnormal	No. services	Breeding efficiency
	(mo.)		*(ml.)*	*(mill./ml.)*	*(%)*	*(%)*		*(%)*
456B1	15	12	1.8	630	70	23.0	3	66
			Biopsy specimen taken from left testicle					
	17	18	2.1	720	80	25.0	17	53
			2nd biopsy specimen taken from left testicle					
	30	4	3.5	500	60	26.5	5	60
203B1	11	4	1.6	630	55	23.2	2	50
			Biopsy specimen taken from left testicle					
	13	8	2.1	750	75	16.0	9	77
215B1	11	3	1.5	450	35	33.0	4	0
			Biopsy specimen taken from right testicle					
	13	7	1.7	570	55	38.0	3	0
29B1	11	9	2.5	570	55	22.0	9	55
			Biopsy specimen taken from right testicle					
	13	10	1.4	660	85	21.5	44	59
264B1	10	14	1.9	390	35	26.0	3	33
			Biopsy specimen taken from right testicle					
	13	5	1.5	660	85	20.4	22	59
210B2	12		Unable to get collection					
			Biopsy specimen taken from left testicle					
	16	16	2.2	570	55	33.4	4	75
	21	11	2.4	715	60	30.0	7	57
			2nd biopsy specimen taken from left testicle					
	24	4	2.5	700	60	31.3	5	80
359B1	12	11	2.6	1,120	78	23.4	5	60
			Biopsy of both testicles simultaneously					
	15	12	2.9	1,540	85	24.4	19	89
456B2	12	15	1.8	780	41	18.4	5	40
			Biopsy of both testicles simultaneously					
	18	25	1.7	0	0	0	0	0
493B2	11	8	2.0	670	70	22.4	8	50
			Biopsy specimen taken from left testicle					
	16	16	2.4	720	78	21.7	7	57
493B3	11	8	1.8	560	74	18.2	10	60
			Biopsy specimen taken from left testicle					
	16	16	2.6	800	77	22.3	14	57

preciably, except for bull 456B2. Bull 215B1 was used in service only seven times and was not successful in getting a cow pregnant. When the biopsy specimen of this bull's testicle was examined, a distinct lack of spermatogenesis was noted with very few spermatids and considerable distortion of the basement cells of Sertoli. After the bull was slaughtered, the histological picture of the testicle had not altered. There was evidence of scar tissue near the site of the biopsy of the right testicle. The remainder of the testicle, however, showed no effect of the biopsy.

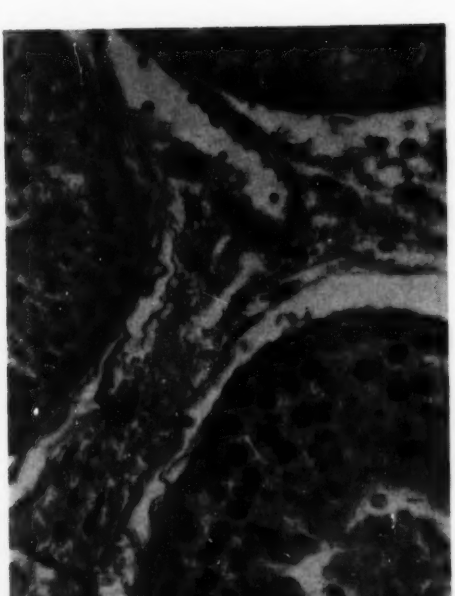

FIG. 1. 210B2, first biopsy left testicle, H and E, 450X.

FIG. 2. 210B2, second biopsy left testicle, H and E, 450X.

Figures 1 and 2 show the testicle of bull 210B2 at the first and at the second biopsy. The similarity between the two sections is evident. The histological analysis of the Holstein bull 456B1, which was biopsied twice in the same testicle, showed the same result as that of bull 210B2.

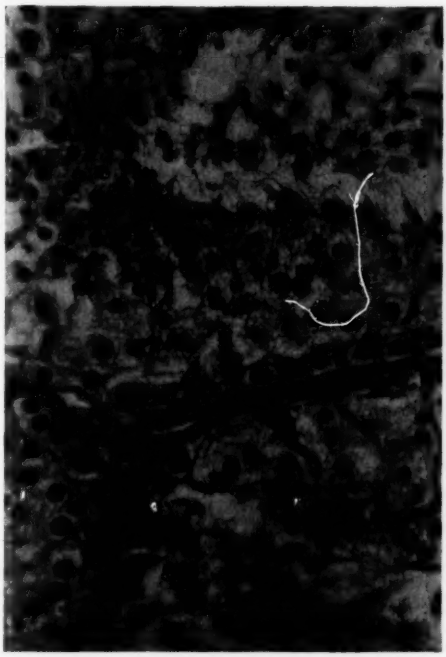

FIG. 3. 359B1, biopsy left testicle, H and E, 450X.

Bull 359B1, which had been biopsied in both testicles simultaneously, showed only a small necrotic area about the site of the biopsy when slaughtered. Figure 3 shows the biopsy specimen and Figure 4 shows the slaughter specimen. When bull 456B2, treated similarly to 359B1, was slaughtered, both testicles showed connective tissue invasion and nearly all seminiferous tubules obliterated. The necrosis had been practically complete.

DISCUSSION

It is evident from the data presented that biopsy of testicles of bulls ranging in age from 8 to 36 months caused no appreciable effect on semen quality. The biopsy is simple to perform and the bull recovers rapidly. The technique allows study of the effect of a variety of treatments within the life span of an individual bull. It was possible to determine that bull 215B1 would not have a high breeding efficiency, since testicular development was very limited, and therefore would not be a sound economic investment. On slaughter of the animal, the bi-

opsied testicle showed scar tissue at the site of the biopsy and an adhesion of the tunica albuginia with the tunica vaginalis. This was true of all bulls biopsied and slaughtered. The remainder of the testicle, however, showed no deleterious effect from the biopsy.

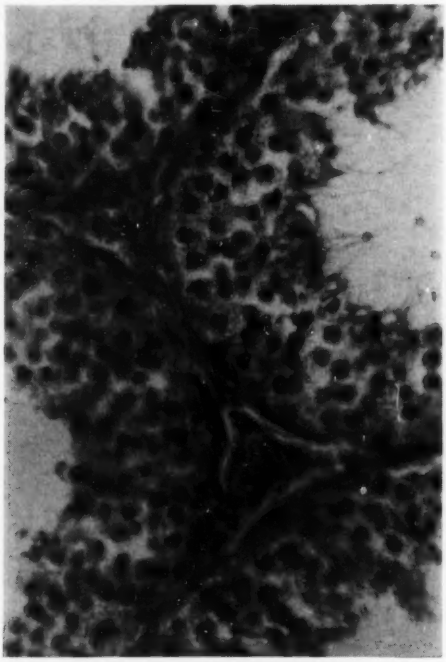

FIG. 4. 359B1, slaughter specimen left testicle, H and E, 450X.

It would appear, in view of the number of successful operations, that some error in technique was committed in bull 456B2, resulting in his complete sterility. After the animal was slaughtered, no evidence as to what this error may have been was observed. This bull did show, however, a large abscessed kidney, three times the normal size. In addition, the peritoneum was adhered to the abdominal walls. This obvious toxic condition of apparent long standing may have retarded recovery from the biopsy operation.

Bulls 456B1 and 210B2 were biopsied for a second time in the same testicle at the ages of 2½ and 2 years, respectively. Although the second biopsy lowered the quality of their semen with respect to total spermatozoa and, in bull 456B1, the motility, the difference is not great. The histological picture of the specimens of the first and second biopsies shows no marked difference, as seen in Figures 1 and 2. Bull 456B1 was bred by artificial insemination to three cows, resulting in three pregnancies after five services. Bull 210B2, by natural service, settled four out of five cows after the second biopsy. Both of these bulls are now in natural service in farm herds.

It has been possible through the biopsy technique to follow the development of the testicles of respective bulls. Since it is believed that the technique itself does not appreciably influence the breeding efficiency of the bulls when they range in age from 8 months to 3 years, it is concluded that a variety of treatments can be studied on each of many bulls.

Although this work does not agree with that of Sykes *et al.* (3), who concluded that the actual biopsy of the testicle influences semen quality, it is believed that in this age group the actual breeding efficiency of the bulls has not been reduced. It would be desirable to have additional data with mature bulls. However, it is contended that where a variety of treatments are to be evaluated, it is with the younger bulls that these treatments are concerned. It is suggested that in addition to testing the effect of treatments on the development of a testicle, the biopsy technique allows the evaluation of a bull's capacity to produce high quality semen.

SUMMARY

Ten bulls of the Jersey and Holstein breeds were biopsied, with one Jersey and one Holstein being biopsied in the same testicle twice and two Holsteins being biopsied simultaneously in both testicles. On the average, semen quality was not appreciably lowered as a result of the biopsy.

One bull biopsied once in only one testicle showed only a small amount of scar tissue at the actual biopsy site when slaughtered 6 months later. A second bull, which had been biopsied on both testicles simultaneously, showed complete necrosis of both testicles on slaughter 6 months later. A third bull, treated similarly to the second showed, when slaughtered 15 months later, only a small necrotic area about the actual biopsy sites. Examination of tissue secured on a second biopsy of the same testicle from two bulls showed no change histologically from that of the first biopsy.

In view of the success of the biopsy technique with nine bulls, it is suggested that study of a variety of treatments with the same bull is possible and an evaluation of a bull's ability to produce high quality semen may be made.

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THE TOXAPHENE AND CHLORDANE CONTENT OF MILK FROM COWS RECEIVING THESE MATERIALS IN THEIR FEED

R. H. CARTER, P. E. HUBANKS, AND F. W. POOS
Bureau of Entomology and Plant Quarantine

AND

L. A. MOORE AND RAY E. ELY
Bureau of Dairy Industry, U. S. Department of Agriculture
Washington, D. C.

Toxaphene and chlordane have been widely used in the control of insects attacking forage crops such as alfalfa, clover, peanuts, and range grass, and other crops such as corn and peas, parts or all of which eventually are used as animal feed. The feeding of forage crops containing residues of some insecticides to milk cows has been found to result in elimination of the insecticide in the milk. In June 1950, Roark (8) published a digest of information on toxaphene and in April 1951 (9) a digest of information on chlordane. Each of these digests contains a discussion of the effects of these chemicals on animals, as well as references to the literature on the subject.

Bushland *et al.* (1) give data on the amounts of toxaphene, chlordane, and other insecticides found in the fat of sheep, goats, and beef after repeated sprayings with these materials. Radeleff *et al.* (7) reported that repeated spraying of Hereford steers with 0.5% toxaphene did not result in the accumulation of large amounts of toxaphene in the body fat. Knipling (6) discussed the toxaphene and chlordane content of milk and fat samples from animals which had been treated with these materials for the control of external parasites. Ely *et al.* (5) have reported on the toxaphene and chlordane content of milk as affected by feeding alfalfa hay sprayed with these insecticides. Further information on the effects of feeding to milk cows alfalfa hay containing toxaphene and chlordane residues and of administering these materials by other methods is presented in this paper.

EXPERIMENTAL PROCEDURE

In July 1948, alfalfa was sprayed under normal field conditions with a toxaphene emulsion at the rate of 1.5 lb. of toxaphene per acre. The spray was applied with a weed sprayer which delivered about 5 gal. per acre at a pressure of 40 lb. per square inch. A chlordane emulsion was applied on the same date and under the same conditions at a dosage of 1.0 lb. per acre. Three days later the hay was cut, field baled, and stored until fed to six milking cows from December 6, 1948, until May 4, 1949.

In May 1949, alfalfa was sprayed with emulsion sprays of toxaphene at the rate of 1.5 lb. per acre and chlordane at a rate of 2.0 lb. per acre. This hay was cut one week later, field baled, and stored until fed to six milking cows from September 2 to December 8, 1949.

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A sample of hay was taken for analysis from each bale fed and composited for each 10-day period of feeding. The method of analysis has been described by Carter and Hubanks (3). The amounts of residue found on the hay samples are shown in Table 1. The p.p.m. of insecticide were calculated from the total organic chlorine content of the hay samples after correction for the amount found for similar determinations on untreated alfalfa.

The animals used for these tests were normal healthy milking cows. Live weights of the animals were recorded on two successive days at 10-day intervals. All animals were fed a few pounds of hay daily in excess of their average daily consumption. Daily records were kept of the amounts fed and refused and the amounts of milk produced. Varying amounts of a (milking-herd) grain mixture were fed, depending on the level of milk production. An aliquot of the milk produced by each animal for 2 days during each 10-day period was taken for analysis. A summary of the averages of the amounts found in these milk samples is given in Table 2.

At the end of the hay feeding tests on December 8, 1949, the same cows were fed technical grade toxaphene and chlordane in soybean oil solution at different levels of intake. Samples of the milk were taken as previously described for analysis. While the oil solutions were being fed, the cows were

TABLE 1

Toxaphene and chlordane residues on alfalfa hay used in feeding tests

Date	Toxaphene	Chlordane
	(p.p.m.)	*(p.p.m.)*
December 10, 1948	58.7	.....
20	99.7	14.0
30	71.7	20.3
January 9, 1949	139.7	38.2
19	.....	46.2
29	55.8	.....
February 8	158.6	15.3
18	60.7	10.1
28	48.5	9.6
Mareh 10	65.4	4.9
20	71.5	.....
30	48.5	8.4
April 9	.....	19.7
19	82.3	.....
29	.....	38.0
av.	81.8	20.4
September 6, 1949	.....	14.7
16	6.2	3.1
26	105.0	11.0
Oetober 6	70.4	1.9
16	70.0	1.4
26	2.4	1.8
November 5	1.3	21.0
15	15.0	92.3
25	23.3	4.6
Decemher 5	12.0	36.0
8	12.6	.....
av.	31.8	20.8

TABLE 2

Toxaphene and chlordane content of milk samples from cows receiving these insecticide residues on alfalfa hay

Cow No.	Days of test	Insecticide residues: Material	On hay av.	Av. daily intake		Av. insecticide in FCM	Av. daily production
			(p.p.m.)	*(mg.)*	*(mg./kg. of body wt.)*	*(p.p.m.)*	*(lb.)*
279	150	Toxaphene	81.8	762	1.63	0.5	22.8
623	150	"	81.8	491	1.37	0.5	18.0
624	150	"	81.8	586	1.70	0.6	25.6
282	100	"	31.8	243	0.45	0.1	31.4
284	100	"	31.8	215	0.51	0.1	26.8
630	100	"	31.8	163	0.51	0.1	23.7
274	150	Chlordane	20.4	201	0.36	0.1	21.2
622	150	"	20.4	155	0.42	0.2	25.7
626	150	"	20.4	118	0.40	0.2	23.2
144	100	"	20.8	162	0.34	0.0	21.7
328	100	"	20.8	139	0.43	0.0	15.3
331	100	"	20.8	137	0.36	0.0	25.7

fed a control hay which contained no insecticide residue. The amounts of oil solution administered, the number of days, and the average amounts of the insecticides found in the milk are shown in Table 3. The relationship between the daily intake of insecticides and the amount recovered in the milk is shown in Figure 1. The organic chlorine content of the milk was determined by the

TABLE 3

Toxaphene and chlordane content of milk samples from cows receiving these insecticides in oil solutions

Cow No.	Days of test	Material	Daily intake of insecticide[a]		Av. insecticide content in FCM	Av. daily production
			(mg.)	*(mg./kg. of body wt.)*	*(p.p.m.)*	*(lb.)*
630	88	Toxaphene	50	0.15	0.13	21.5
	45	"	1000	2.92	0.50	19.0
	61	"	3000	8.15	1.98	13.3
282	88	"	200	0.36	0.11	28.8
	45	"	1500	2.68	0.72	28.2
	71	"	4000	6.68	2.34	25.6
284	88	"	500	1.14	0.00	25.4
	45	"	2000	4.44	0.75	23.4
	71	"	5000	9.32	3.39	21.7
328	88	Chlordane	50	0.14	0.08	13.8
	45	"	1000	2.78	0.57	13.1
	71	"	3000	8.31	5.16	11.2
331	88	"	200	0.52	0.11	20.9
	45	"	1500	3.63	0.80	21.5
	71	"	4000	9.83	7.95	20.8
144	88	"	500	0.92	0.20	21.6
	45	"	2000	3.60	0.98	20.4
	71	"	5000	9.01	7.34	15.2

[a] 50 to 2,000 mg. given in oil solution in capsules; 3,000, 4,000, and 5,000 mg. given in oil solution mixed with grain.

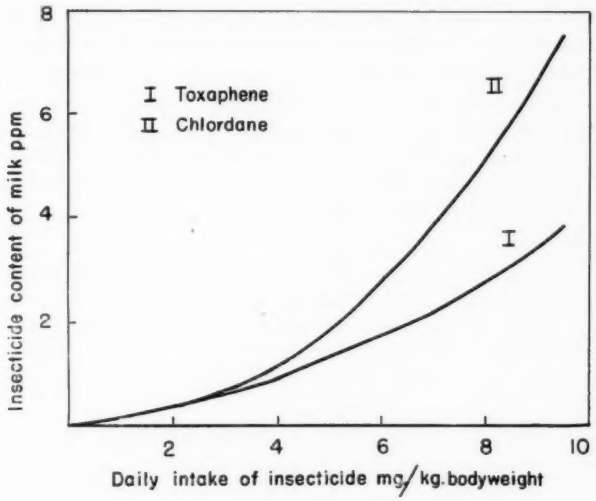

FIGURE 1

method described by Carter (2). Corresponding determinations made on control milk samples at the same time were used to correct the results obtained on the milk from the cows on the test. An average of 0.8 p.p.m. organic chlorine was found in 23 check determinations on control milk samples taken throughout the course of the test from regular herd production.

RESULTS AND DISCUSSION

A summary of the analyses of the milk samples from the cows receiving insecticide residues on hay is given in Table 2 and of the milk samples from the cows receiving toxaphene and chlordane in oil solutions in Table 3. The results are calculated on the basis of fat-corrected milk.

The data in Table 2 indicate that a daily intake of either toxaphene or chlordane residues below approximately 0.5 mg. per kilogram of body weight did not result in the excretion of accurately measurable amounts of these compounds in the milk judged by organic chlorine determinations. The data in Table 3 also indicate that daily intakes of 0.3 to 0.5 mg. per kilogram body weight of toxaphene and chlordane in oil solutions did not measurably increase the organic chlorine content of the milk. A daily intake of 1.4 mg. of toxaphene per kilogram of body weight did result in an apparent toxaphene content of the milk of 0.5 to 0.6 p.p.m. Residues of chlordane on the hay were not high enough to reach this level of intake.

The data in Table 3 indicate that daily intake up to 4 mg. per kilogram of body weight of toxaphene and chlordane did not result in the excretion of these compounds in amounts in excess of 1 p.p.m. Approximately 9 mg. per kilo-

gram of body weight of toxaphene and chlordane resulted in the elimination of 3 to 4 and 7 to 8 p.p.m., respectively, of these compounds. In Figure 1 the daily intakes of toxaphene and chlordane in milligrams per kilogram of body weight are plotted against the p.p.m. of the insecticide found in the milk. Up to an intake of approximately 2.0 mg. per kilogram of body weight the amounts found in the milk were of the same order of magnitude (below 0.5 p.p.m.), irrespective of the method of administration or the compound ingested. At intakes above 3 mg. per kilogram there was apparently more chlordane than toxaphene excreted in the milk.

An insecticide residue of 160 p.p.m. would be required to supply 4 mg. per kilogram of body weight for an 800-lb. cow consuming 20 lb. of hay daily. Diephuis and Dunn (4) have reported toxaphene residues of 25-40, 81, and 306 p.p.m. on alfalfa hay which had been treated with 2, 4, and 8 lb., respectively. The alfalfa had been treated during the summer and the hay samples were analyzed during the winter months.

After the intake of toxaphene and chlordane had been discontinued for 30 days the organic chlorine content of the milk had returned to normal.

SUMMARY

Feeding tests were conducted in which toxaphene and chlordane as residues on alfalfa hay and in soybean oil solutions were included in the daily ration of milk cows.

The toxaphene residues on the baled alfalfa as fed averaged 81.8 and 31.8 p.p.m. for the 1948 and 1949 crops, respectively, which had been sprayed with 1.5 lb. per acre. The chlordane residues on the baled alfalfa averaged 20.4 and 20.8 p.p.m. for the 1948 and 1949 crops which had been treated with 1.0 lb. and 2.0 lb. per acre, respectively. The 1948 crop was fed from December 6, 1948, to May 4, 1949; the 1949 crop from September 2 to December 8, 1949.

A daily intake of toxaphene for 150 days as a residue on alfalfa hay of 586 to 762 mg. (1.70 to 1.63 mg. per kilogram of body weight) resulted in an apparent toxaphene content in the milk of approximately 0.5 p.p.m. A daily intake for 100 days of 163 to 243 mg. (0.45 to 0.51 mg. per kilogram of body weight) resulted in an apparent toxaphene content of 0.1 p.p.m. in the milk.

A daily intake of chlordane for 150 days as a residue on alfalfa hay of 118 to 201 mg. (0.36 to 0.42 mg. per kilogram of body weight) resulted in an apparent chlordane content of 0.1 to 0.2 p.p.m. in the milk. A daily intake for 100 days of 137 to 162 mg. (0.34 to 0.43 mg. per kilogram of body weight) did not result in a measurable amount of chlordane being eliminated in the milk.

An intake up to 2,000 mg. daily of toxaphene in oil solution (2.7 to 4.4 mg. per kilogram of body weight) resulted in an apparent toxaphene content of less than 1.0 p.p.m. in the milk. A maximum apparent toxaphene content of 3.4 p.p.m. resulted from a daily intake of 5,000 mg. (9.3 mg. per kilogram).

An intake up to 2,000 mg. daily of chlordane in oil solution (3.6 mg. per kilogram body weight in two cases) resulted in an apparent chlordane content of approximately 1.0 p.p.m. in the milk. An apparent chlordane content of 8 p.p.m.

resulted from a daily intake of 4,000 mg. (9.8 mg. per kilogram of body weight) and of 7.0 p.p.m. from 5,000 mg. (9.0 mg. per kilogram of body weight).

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THE EFFECT OF PREPARTUM MILKING UPON THE QUANTITY OF MILK PRODUCED DURING THE SUBSEQUENT LACTATION[1]

ERVIN P. SMITH AND EVERETT A. KEYES
Department of Dairy Industry,
Montana Agricultural Experiment Station
Bozeman

There is a lack of agreement among research workers as to the effectiveness of prepartum milking as a means of reducing mammary and umbilical edema often present at parturition. Some authors (2, 3, 9, 14) have proposed prepartum milking as a means of relieving edema. Other investigators (1, 5) have reported that prepartum milking did not affect significantly the mammary and umbilical edema present at parturition. In contrast, Smith and Keyes (12) found that prepartum milking relieved udder congestion in Holstein cows, but not in Jersey cows. If prepartum milking would relieve congestion in the udder and condition it for normal milk flow, the practice should be conducive to increased milk production for the following lactation period. Keyes and coworkers (8) found that prepartum milked cows produced more milk on the 4th day postpartum than did cows that were not prepartum milked. Dawdy and Knodt (4) determined the effect of prepartum milking on milk production by prepartum milking one half of the udder and not prepartum milking the other half. These workers found that prepartum milked halves averaged 5,061 lb. of milk, and the halves not prepartum milked averaged 4,780 lb. This is an average difference of 280 lb. of milk in favor of the prepartum milked halves for the first 7 months of lactation. Gaunya and Eaton (7) found that there was no significant difference in the yields of fat corrected milk in comparing 22 prepartum milked cows with a similar group of 22 postpartum milked cows. These same investigators found no significant difference when the yields of 21 prepartum milked cows were compared with the yields of a subsequent or previous lactation.

Because of the diversity of reports on the effects of prepartum milking, it was the purpose of this experiment to further determine if the prepartum milked cows would produce a larger quantity of milk during the subsequent lactation than the cows that were not prepartum milked.

EXPERIMENTAL

Forty-seven cows and heifers due to freshen were divided into four groups. The cows within each breed were assigned to a prepartum milked group or a check group at random. Group I, consisting of 19 Holstein cows, was a prepartum milked group, and Group II, consisting of 8 Holstein cows, was a check group.

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Group III, consisting of 13 Jersey cows, was a prepartum milked group, and Group IV, consisting of 7 Jersey cows, was a check group. All the cows and heifers in the experiment were put in a box stall in the barn 7 days before the calculated freshening date so they would become acquainted with their surroundings and would be available for milking. Each cow was fed 3 lb. of the herd concentrate mix at each milking. The concentrate mix for the years 1946 to 1949 was as follows:

Barley	385 lb.
Bran	200 lb.
Beet pulp	300 lb.
Soybean meal	100 lb.
Bone meal	5 lb.
Salt	10 lb.
Total	1000 lb.

The protein content of the herd concentrate mixture given above was found by analysis to be 16.6%.

The concentrate mixture for the year 1949 to 1950 was as follows:

Barley	285 lb.
Bran	200 lb.
Beet pulp	325 lb.
Soybean meal	175 lb.
Bone meal	5 lb.
Salt	10 lb.
Total	1000 lb.

The protein content of the herd concentrate mixture given above was found by analysis to be 19.4%. All cows and heifers were fed second cutting alfalfa of good quality. Immediately after parturition, each cow was given a warm bran mash and all the warm water she would drink.

The prepartum milked cows were milked twice a day, beginning the 7th day before the calculated freshening date. The actual number of days the cows in Group I (Holsteins) were prepartum milked ranged from 2 to 10 and averaged 6.4. The actual number of days those in Group II (Jerseys) were prepartum milked ranged from 2 to 10 and averaged 6.7. All cows were milked twice daily 4 days postpartum. All the cows were transferred to the regular milking herd on the 5th day postpartum and were milked three times daily for the remainder of the lactation period.

The milk obtained from each cow was weighed at each milking, and a record was kept of the daily quantity produced for the first 305 days. The fat percentage determined by the Babcock method by the HIR tester was used as the

average per cent fat. The monthly fat yield was calculated using that percentage. The milk yield was converted to fat-corrected milk by use of the following formula given by Petersen (9):

$$\text{FCM} = 0.4 \times \text{yearly milk production} + 15 \times \text{yearly fat production}$$

The data obtained were analyzed according to statistical procedures (13).

RESULTS

Thirty-three cows completed 305 days of lactation following the prepartum milking experiments. The average amount of fat-corrected milk produced by the prepartum milked Holsteins in Group I was 13,960 ± 2,111 lb., compared with the average of 14,362 ± 3,754 lb. produced by the check Holsteins (Group II). The prepartum milked Jerseys, Group III, produced an average of 9,529 ± 814 lb. of fat-corrected milk compared with an average of 11,111 ± 814 lb. produced by Group IV, the check Jersey group. The data were analyzed by the analysis of variance (13), which indicated that there was no significant difference in production between cows prepartum milked and those not milked prepartum.

SUMMARY AND CONCLUSIONS

Of 47 Holstein and Jersey cows and heifers used in a prepartum milking experiment 33 completed 305 days of lactation following the experiment. The average amount of fat-corrected milk produced by 13 prepartum milked Holsteins was 13,960 ± 2,111 lb., compared with an average of 14,362 ± 3,754 lb. produced by five Holsteins that were not milked prepartum. The average amount of fat-corrected milk produced by 10 prepartum milked Jerseys was 9,529 ± 1,840 lb., compared to the average of 11,111 ± 814 lb. produced by five Jerseys that were not milked prepartum. When the data were analyzed by analysis of variance, the differences were not found to be significant. It was concluded that, under the conditions of this experiment, prepartum milking did not result in an increased quantity of milk produced in the lactation which followed prepartum milking.

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DECLINE IN FERTILITY OF BULL SEMEN WITH INCREASE IN STORAGE TIME AS INFLUENCED BY DILUTION RATE

E. L. WILLETT

American Foundation for the Study of Genetics, Madison, Wisconsin

That breeding efficiency is reduced when spermatozoan numbers decline below a total of 12 million (10, 14, 16) or below 12 million motile spermatozoa (2) has been demonstrated. Various workers (1, 6, 8, 11, 13, 14) have shown that motility of spermatozoa decreases more rapidly during storage with increase in extension rate. On the other hand, Melrose (7) recently presented data which indicated that decline in fertility with increase in time of storage of bull semen is not influenced by dilution. He compared extension rates of 1:20 and 1:50 and of 1:50 and 1:100 and observed no significant differences in nonreturn rates in these two comparisons. Salisbury (9) compared extension rates of 1:40, 1:60, 1:80, and 1:100 and also observed no significant differences in fertility. In light of these facts, one might be more likely to obtain differences in rate of decline of fertility as time of storage is increased if the semen is extended 1:100 and 1:300. Willett and Larson (16) have demonstrated highly significant differences in fertility between these dilution levels. For this reason, three experiments have been conducted comparing fertility of semen extended at these two rates and used to breed cows on the day the semen is collected and the day following.

EXPERIMENTAL

In every experiment the semen was collected early in the morning. Breedings were performed only by technicians located close to the source of semen because these men were able to breed cows with semen on the same day it was collected (first day) and also the day following (second day). The dilution levels of 1:100 and 1:300 were alternated with successive collections from individual bulls. Limited studies by the author (15) have indicated that a split-collection experimental design with less than 30 breedings per sample is less efficient than one in which treatments are alternated with successive collections from the same bull. Comparison of first-day with second-day breedings can, however, be considered as being on a split-collection basis because in each case cows were bred on both days with semen from the same collections. Two or more tubes of semen were given each technician in order to avoid breeding cows on the second day with semen that had been carried in the field during the preceding day. Fertility was measured by 60- to 90-day nonreturns from first services in the first two trials and, in order to obtain an increased number of breedings, first and second services in the third trial.

In the first experiment, 24 technicians associated with four studs bred 1,382

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cows with semen from 233 collections from 49 bulls during the months of August, September, and October. The number of services per collection varied from 5 to 38. Comparable data for the second trial were eight technicians, 1,992 services, 108 collections, 14 bulls, April, May, and June, and 4 to 39 services per collection. Similar data for the third experiment were seven technicians, 3,233 services, 132 collections, 18 bulls, May to August, inclusive, and 5 to 48 services per collection. Unless each bull and technician were represented in all four treatments or unless each collection was represented in both first and second days, the data were not included in these studies. In each experiment bulls of the Guernsey, Holstein, and Jersey breeds were represented. Four bull studs were involved in the first trial and one in the two later experiments. The experiments were conducted in successive years.

RESULTS AND DISCUSSION

The results, based upon 6,607 services, are summarized in Table 1. The average nonreturn rate for the dilution level of 1:100 was 68.2% and for 1:300, 62.4. The difference of 5.8 percentage units is highly significant ($P < 0.001$). The average for the first day was 68.4% and for the second day, 62.5. This difference of 5.9 percentage units is also highly significant ($P < 0.001$).

TABLE 1

60- to 90-day nonreturns from first-day and second-day breedings with semen extended 1:100 and 1:300

Experiment	Day	1:100		1:300		Nonreturn rate differences
		Services	Nonreturns	Services	Nonreturns	
		(No.)	*(%)*	*(No.)*	*(%)*	
I	1st	324	72.2	311	68.2	4.0
	2nd	375	70.4	372	61.8	8.6
Totals & avs.		699	71.2	683	64.7	6.5
Decline from 1st to 2nd day			1.8		6.4	4.6
II	1st	473	71.0	482	69.1	1.9
	2nd	538	72.1	499	65.5	6.6
Totals & avs.		1,011	71.6	981	67.3	4.3
Decline from 1st to 2nd day			—1.1		3.6	4.7
III	1st	793	68.6	790	64.9	3.7
	2nd	784	60.7	866	53.2	7.5
Totals & avs.		1,577	64.7	1,656	58.6	6.1
Decline from 1st to 2nd day			7.9		11.7	3.8
All	1st	1,590	70.1	1,583	66.8	3.3
	2nd	1,697	66.5	1,737	58.6	7.9
Totals & avs.		3,287	68.2	3,320	62.4	5.8
Decline from 1st to 2nd day			3.6		8.2	4.6

For all experiments combined, the average decline in nonreturn percentage units from first day to second day with semen diluted 1:100 was 3.6, and with 1:300, 8.2. The difference in decline was 4.6. The differences in rates of decline were consistent from experiment to experiment and for the three experiments were 4.6, 4.7, and 3.8 percentage units, respectively. The combined data for the three experiments were analyzed by means of a $2 \times 2 \times 2$ chi-square table (12). The interaction between dilution level and time of storage is short of significance ($P = 0.1$) (5). These data based upon fertility, however, parallel those based upon motility of spermatozoa (1, 6, 8, 11, 13, 14). The fertility data also support the observations of other workers (3, 4), who noted that high dilutions have a direct and detrimental effect upon spermatozoa.

SUMMARY

Three experiments involving 6,607 services have been conducted to compare rate of decline in nonreturn rate from day of collection to the next day with semen extended 1:100 and 1:300. The average nonreturn rate for semen diluted 1:100 was 68.2% and for semen diluted 1:300, 62.4%. The difference of 5.8 percentage units is highly significant. The average nonreturn rate with semen used on the day of collection was 68.4% and on the next day was 62.5%. This difference of 5.9 percentage units is also highly significant.

The average decline from day of collection to the next day with 1:100 was 3.6% and with 1:300 was 8.2%. The differences in decline with 1:100 and with 1:300 were quite consistent in the three experiments. The interaction of age and dilution level was short of significance ($P = 0.1$), however. The breeding results parallel motility observations reported in the literature and support other work which indicates that high dilutions have a direct, detrimental effect upon spermatozoa.

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EFFECT OF TRANSPORTATION UPON FERTILITY OF DAIRY BULLS

E. L. WILLETT AND G. L. LARSON
American Foundation for the Study of Genetics, Madison, Wisconsin

The common belief among dairymen has been that the fertility of bulls is sometimes impaired by their transportation. Text books often recommend that precautions be taken, including the use of a suspensory support for the testicles. A survey of the literature produced little evidence supporting this belief or such a recommendation.

Dawson (2) studied the conception rates of 17 bulls after they had been transported varying distances. He stated: "While the average fertility is decidedly downward for the first year following movement of the sires and is decidedly upward for the second year, the individual variation is so wide that definite conclusions cannot be drawn." There were 1,406 services during the 2-year periods studied. Recently, however, Meschaks (3) has reported sterility in one bull and a temporary increase in excretion of neutral steroids in the urine of three bulls after moving. He attributed this high steroid excretion to transport and termed the condition "transport stress."

In a recent personal communication, Arnold (1) has reported studying the records of 4,000 bulls in natural service. According to owners' reports, 27 (0.68%) "went sterile when shipped." The number of shipments or type of transportation was not recorded nor could it be determined from the records whether or not the sterility was a primary or a secondary result of the shipping. In none of the completed records of 300 bulls used artificially did Arnold encounter a case of sterility attributed specifically to transportation.

To obtain information concerning the effect of transportation on fertility of bulls, the authors have studied records collected in a large artificial breeding organization which has a number of separate studs between which bulls are moved periodically to avoid inbreeding. This paper presents data concerning some of the semen characteristics and fertility of these bulls before and after moving.

EXPERIMENTAL

The data used in this study were from bulls which were moved by truck or railroad at all seasons of the year among four different studs over distances varying from approximately 300 to 900 miles. In no case were the testicles protected by suspensory supports. Sixty- to ninety-day nonreturns to first services performed the day following collection of semen, spermatozoan concentrations in undiluted and in diluted semen, and semen volumes were compiled for individual bulls for the 3 months prior to moving and for each of the 2 months following moving. If the time from the first of the month to the first day of moving was

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less than 15 days, the data were added to that from the previous month and considered as the month preceding moving. Likewise, if the time from the first day of moving to the end of the month was less than 15 days, the data were added to that from the following month and termed the month following moving. In many cases, the number of services in these fractional months was small. The "month following moving," therefore, actually covered a period varying from about 2 to 6 weeks. In instances where data for a bull included less than 30 services in the 3-month period prior to moving or in one of the two periods following moving, those data were excluded from this study. Only 24 of the 108 items remaining were based on less than 100 services. Spermatozoan numbers in semen were determined electrometrically (4). This study covers a total period of 38 months. One Angus, 2 Brown Swiss, 11 Guernsey, 12 Holstein, and 10 Jersey, a total of 36, bulls are represented in the data.

RESULTS AND DISCUSSION

The average nonreturn rates, semen volumes, and numbers of spermatozoa per milliliter and per ejaculate for each of the 3 months before moving were almost identical. Average figures for the 3 months for each bull were therefore calculated and considered to be the best estimates for each bull prior to moving. The data for the 3-month period prior to moving and for each of the 2 months following moving are summarized in Table 1. The results were fairly consistent

TABLE 1

Fertility and semen characteristics of bulls before and after moving

Breed	Aberdeen Angus	Brown Swiss	Guernsey	Holstein	Jersey	All breeds
Bulls *(No.)*	1	2	11	12	10	36
Before moving						
Ejaculates *(No.)*	20	23	297	271	226	837
Vol./ejaculate *(ml.)*	3.2	5.4	6.1	6.2	4.3	5.5
Sperm/ml., fresh *(millions)*	2,104	1,354	1,568	1,443	1,686	1,549
Sperm/ejaculate *(millions)*	6,733	7,312	9,565	8,947	7,250	8,599
Dilution rate *(1:)*	146	116	121	132	115	124
Services *(No.)*	142	211	10,129	18,807	3,845	33,134
Non-returns *(%)*	64.8	64.4	63.9	68.5	66.6	66.8
1st mo. after moving						
Ejaculates *(No.)*	5	12	68	84	54	223
Vol./ejaculate *(ml.)*	4.7	7.3	6.5	6.4	5.1	6.1
Sperm/ml., fresh *(millions)*	2,330	1,514	1,269	1,340	1,642	1,406
Sperm/ejaculate *(millions)*	10,951	11,052	8,248	8,576	8,374	8,590
Dilution rate *(1:)*	109	125	120	122	112	119
Services *(No.)*	67	129	2,883	5,556	1,643	10,278
Non-returns *(%)*	67.2	71.3	67.0	70.8	67.1	69.2
2nd mo. after moving						
Ejaculates *(No.)*	6	11	75	96	67	255
Vol./ejaculate *(ml.)*	5.2	8.1	6.9	6.6	4.9	6.3
Sperm/ml., fresh *(millions)*	2,245	1,594	1,462	1,397	1,494	1,466
Sperm/ejaculate *(millions)*	11,674	12,911	10,088	9,220	7,321	9,189
Dilution rate *(1:)*	82	101	137	130	115	127
Services *(No.)*	65	113	4,279	7,254	1,823	13,534
Non-returns *(%)*	76.9	70.8	65.2	70.1	68.7	68.4

from breed to breed. Twelve bulls, however, had a higher nonreturn rate before moving than during the first month after moving, 22 had a higher nonreturn rate after moving, and two had no change. The average fertility of the bulls was slightly higher after moving than it was before. The average nonreturn rate for each of the 2 months following moving or for the 2 months combined was not significantly greater than the average for the previous 3 months. In each case the mean square for periods was approximately the same as the mean square for error. The average volume of semen per ejaculate during the first and the second months after moving was highly significantly greater ($P < 0.01$) than the volume before moving. However, the number of spermatozoa per milliliter of ejaculated semen was lower after moving, but not significantly so. The net result was that the number of spermatozoa per ejaculate remained almost the same during the period prior to moving and the first month after moving and increased during the second month after moving. This increase was not significant. There was no significant breed and period interaction in any of the analyses of variance made of these data. These data indicate that transportation had not affected the fertility of the bulls studied.

The insignificant increase in fertility of bulls after moving might be explained by observations made in these studs. In a number of instances the libido of bulls prior to moving became so reduced that the usefulness of the bulls was seriously impaired. After moving to a new location a marked improvement often occurred. In light of these experiences, it has become the practice, sometimes, to move bulls with reduced libido from one stud to another with frequent beneficial results.

At some time prior to the period studied in this investigation, each bull had been transported for some distance, once to the stud following purchase and also in a few instances once again from one stud to another. The policy has been to move bulls at intervals of 2½ years; therefore, no bull had been transported for at least 2 years prior to the period studied. The question might arise as to whether or not bulls susceptible to the effects of transportation may have been eliminated at the time of the earlier movings. The ones included in this study would, therefore, be those that could undergo such treatment without reduction in fertility. This organization has moved most of its bulls long distances to one of its studs following purchase and many between its various studs. In no instance following moving has any form of sterility or reduced fertility developed that could be attributed specifically to transportation. All of these bulls not only have been checked carefully by men in the bull studs but also have been under close supervision of veterinarians.

SUMMARY AND CONCLUSIONS

Data concerning fertility and some semen characteristics of 36 bulls before and after moving from one stud to another have been compiled.

The average 60- to 90-day nonreturn rate from first services performed the day following collection of semen for a 3-month period prior to moving was

66.8%. That for a 2- to 4-week period following moving was 69.2% and for the next month was 68.4%. These differences are not significant. The average volume of semen per ejaculate prior to moving was 5.5 ml. and for the two periods following moving was 6.1 and 6.3 ml., respectively. This increase in volume after moving is highly significant ($P < 0.01$). The average number of spermatozoa per milliliter of semen during these three periods was 1,549, 1,406, and 1,466 million, respectively. These differences are not significant. The total number of spermatozoa per ejaculate varied little from one period to another, being 8,599, 8,590, and 9,189 million, respectively. These differences also are not significant. These data indicate that the fertility of bulls was not impaired by transportation.

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COMPARISONS OF SILAGES STORED IN GAS-TIGHT SILOS AND IN CONVENTIONAL SILOS

J. B. SHEPHERD, C. H. GORDON, H. G. WISEMAN, AND C. G. MELIN
Bureau of Dairy Industry

AND

L. E. CAMPBELL AND G. D. ROANE
Bureau of Plant Industry, Soils and Agricultural Engineering
U. S. Department of Agriculture, Washington, D. C.

The dry matter and nutrient losses which occur in silage during storage are caused by: *(a)* the physiological and microbiological activity (fermentation) which transform a green crop into silage in the absence of air, *(b)* surface spoilage due to heating, molding, and rotting in the presence of air, and *(c)* seepage (effluent) caused by the weight of the crop pressing out excess moisture containing soluble forage nutrients.

Fermentation losses (so-called intangible losses) can be arrived at by determining surface spoilage and seepage losses separately and deducting these from the total losses for the silo. They can also be determined from sample bags buried in the silage where there is little or no seepage. The extent of the losses will vary with stage of maturity and moisture content of the crop, method of filling and sealing the silo, and length of the storage period.

Dry matter losses in top spoilage at Beltsville (unpublished data) range from 4 to 9% in silos 25 ft. high, filled in June and July and fed out the following winter. This is equivalent to losses of only 2 to 4.5% in a silo 40 ft. tall. These silos were topped off with finely chopped fresh green crop thoroughly compacted by tramping and kept packed tight against the wall while settling. Turk *et al.* (13) reported 5.0% and 4.4% top spoilage and 14.1% and 17.4% top and side spoilage combined for two silos. Part of the side spoilage was attributed to the poor condition of the silos.

Data from Beltsville (unpublished), Ohio (11), New Jersey (7), and Oregon (1) indicate that the dry matter content of silage effluent usually will range from 4.5 to 6.5% for soybeans, 6 to 8% for alfalfa, timothy and clover, and other meadow crops, and 7 to 9% for corn. The addition of molasses increases the dry matter content of effluent to 8.5-9.5% for soybeans (Beltsville) and 9.0 to 11.5% for alfalfa (Beltsville and New Jersey).

Seepage flow begins at 65 to 68% moisture, depending upon the depth of the silage, rarely exceeding 3% of the weight and 1% of the dry matter of a 70% moisture crop. McCalmont *et al.*, New Jersey (7), and Otis, Minnesota (10), obtained the following percentage seepage and dry matter losses in seepage, respectively, with alfalfa forage: alone or with phosphoric acid added, 1.8 and 0.45 at 70% moisture; 6.5 and 1.85 at 72% moisture; 16.0 and 5.38 at 77.1%

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moisture; 19.5 and 6.76 at 78% moisture; with molasses added, 14.1 and 5.35 at 72% moisture; 18.1 and 8.17 at 76.6% moisture. Perkins and Pratt (11) pressed 30% seepage and 10% dry matter out of forage crop containing 82% moisture at 8 lb. pressure per square inch. Watson (14) indicates dry matter losses in seepage as high as 10% when the crop is extremely high in moisture, or is subject to leaching by rain (trenches and stacks).

Dry matter losses due to the silage fermentation at Beltsville in 4 ft. × 8 ft. silos (15, 16) and in farm sized silos (unpublished) usually range between 3 and 8% and seldom exceed 10%. The higher losses usually occur over long storage periods and/or with high moisture crops. Losses of similar magnitude, determined by the sample bag technique, are reported by Camburn *et al.* (2, 3) and Newlander *et al.* (8, 9). These figures are higher in some cases than the fermentation "weight losses" reported by Perkins and Pratt (11) for small laboratory silos over short periods.

Total dry matter losses during storage in tower silos at Beltsville (unpublished) for crops ensiled in the summer and fed out the following winter usually range between 10 and 16%, with losses of 20.6 and 23.7% dry matter, respectively, for two silos of high moisture soybean silage. McCalmont *et al.* (7) report total losses of 16 to 23% dry matter. Hodgson and Knott (6) and Turk *et al.* (13) report losses as high as 25% in tower silos that were not tight, with considerable side spoilage.

Previous experiments at Beltsville (12, 15, 16, 17), the Ohio Agricultural Experiment Station (5), and elsewhere indicate that immature, high-protein forages can be stored in conventional tower silos without any added conditioner and with low losses when the following precautions are taken: *(a)* Wilt the forage slightly to a moisture content between 60 and 70%. *(b)* Chop the wilted forage with a fine cutter setting of ¼ to ⅜ in. to promote rapid settling. *(c)* Level off after each load and tramp next to the wall. *(d)* Fill the silo rapidly to keep temperatures low. *(e)* Seal the top of the silo with 4 to 6 ft. of fresh unwilted crop, finely chopped, evenly distributed, and thoroughly tramped. *(f)* Keep the air sealed out by tramping periodically next to the wall while the silage is settling.

Gas-tight silos constructed from steel plates with a glass-like surface and with a mechanism for unloading at the bottom have been available for experimental use at Beltsville since 1950. It was thought likely that the reactions occurring in gas-tight silos would be different in some respects from those occurring in conventional silos, since the amount of oxygen in contact with the silage would be very limited. Experiments were conducted in 1950 and again in 1951 to determine the extent of losses occurring in gas-tight silos compared to conventional tower silos. A progress report of the first experiment of this series was presented at the 1951 annual meeting of the American Dairy Science Association (4).

Experiment I. A comparison of wilted and half-dry alfalfa stored in gas-tight silos.

EXPERIMENTAL PROCEDURES

Alternate windrows of a first cutting of alfalfa-grass mixed forage were stored in two different (14 ft. × 40 ft.) gas-tight silos in June 1950. One month later, without removing any of the first crop top surface material, alternate windrows of a second cutting of alfalfa-grass mixed forage were stored in the two silos. Thus, each silo was filled to about two-thirds of its capacity, without distributing or tramping. The forage stored in one silo was slightly wilted, averaging 36% dry matter. Forage stored in the other silo was field cured further, averaging 54% dry matter.

The forage was weighed, sampled, and analyzed as it was stored in the silo and as it was removed for feeding. Losses of dry matter and various nutrient constituents were calculated from these data for the two types of silage (Table 1).

TABLE 1

Composition and storage losses of alfalfa-grass silages stored in gas-tight silos

Items	Dry matter	Constituents of dry matter							pH
		Protein[a]	Ether extract	Fiber	N-Free extract	Ash	Sugar	Carotene	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(γ/g.)	
		Slightly wilted silage (36% dry matter content)							
Chemical composition									
as stored	36.06	18.78	2.14	27.41	42.68	8.99	3.35	155.4	
as fed	34.41	19.40	3.45	28.87	38.90	9.38	0.83	78.4	4.29
Losses during storage (%)	5.62	2.48	+51.77	0.62	13.97	1.49	76.51	52.43	
		Half-dry silage (54% dry matter content)							
Chemical composition									
as stored	54.02	18.48	2.17	28.78	41.75	8.82	3.24	96.8	
as fed	54.29	18.58	2.48	29.67	40.43	8.86	2.37	34.9	4.71
Losses during storage (%)	1.04	0.49	+12.16	+2.02	4.19	+0.61	27.55	64.38	

[a] Average of protein determined on wet samples and dry samples.

Both silages were fed as the sole roughage to 14 milking cows, to the limit of their appetite, in an 80-day single reversal feeding trial. Grain consisting of six parts, by weight, of ground corn, four parts ground oats, 1% salt and 1% bone meal also was fed. It was apportioned at 10-day intervals at the daily rate of 1 lb. for each 4 lb. of fat corrected milk produced.

RESULTS

Both silages had a good appearance and aroma when removed for feeding. There was no spoilage in either silo on the top surface of the silage or at the junction of the first and second crop silage. There was no mold in either type of silage, with the exception of a small amount of mold that appeared in the half-dry silage when the silo was nearly empty. This small amount of mold was

probably caused by prolonged contact with atmospheric oxygen on some days when the discharge door of the unloader was left open for several hours.

The total dry matter and constituent loss occurring in the wilted silage was about half the 10 to 15% total loss (including top spoilage) usually found at Beltsville in conventional silos for the same type and depth of silage. The fact that there was no spoilage in the gas-tight silo accounts for most of this difference. The low pH of the wilted silage, the nearly complete utilization of reducing sugars, and a considerable decrease in N-free extract are all characteristics of good wilted silage in a conventional silo. Therefore, the fermentation processes may have been much the same in both kinds of silos. However, this took place without distributing or compacting, both of which are necessary in a conventional silo.

The total dry matter and constituent losses occurring in the half-dry silage were only a fraction of that occurring in the wilted silage — and no higher than expected losses for thoroughly dried field-cured hay stored loose in a hay mow. The higher pH, smaller sugar utilization, and smaller decrease in N-free extract all point to the smaller amount of fermentation expected with the lower moisture content. In a conventional silo there would have been more atmospheric oxygen present and, therefore, more heating and molding with larger losses in half-dry silage than in slightly wilted silage.

Because of the shorter field curing period, the amounts of protein, ether extract, and carotene were greater in the slightly wilted silage than in the half-dry silage.

In a series of previous experiments at Beltsville, dry matter losses during the slight field wilting of similar crops stored as silage has averaged about 6%. During the half drying in the field of similar forage for barn hay drying, the dry matter loss has averaged about 13%. Considering these differences in field losses, there is no particular advantage in extending the field curing period beyond the slightly wilted stage.

The results of the feeding trial are shown in Table 2. Both silages were fed to the limits of appetite and both were very palatable. However, the cows ate a little more dry matter per day and per 100 lb. live weight daily of the half-dried silage than they did of the slightly wilted silage. Calculated digestible protein intake was considerably higher, and calculated total digestible nutrient intake was over 20% higher, than the requirements of the Morrison standard. Live weight and milk production were better maintained on the half-dry silage. Differences in average milk production were small. The winter decline in the vitamin A potency of the butterfat produced was smallest on the slightly wilted silage.

Experiment II. A comparison of slightly wilted alfalfa silages stored in a gas-tight silo and in a conventional silo.

EXPERIMENTAL PROCEDURES

Second cutting alfalfa-light grass crop was divided into nine separate blocks and forage from each block stored in mid-July as slightly wilted silage in a

TABLE 2

Results of feeding trial with grass-legume silages stored in gas-tight silos

Items compared	Slightly wilted silage	Half-dry silage
Live wt. of cows:		
Initial wt. per cow *(lb.)*	1,236.5	1,228.5
Av. wt. per cow *(lb.)*	1,226.7	1,229.9
Av. gain or loss per cow per day *(lb.)*	—0.33	+0.25
Milk production (FCM):		
Initial per cow *(lb.)*	30.75	30.25
Av. per cow per day *(lb.)*	28.29	28.62
30-day decline *(lb.)*	3.5	2.6
30-day decline *(%)*	11.4	8.0
Feed dry matter consumed per cow per day:		
Silage *(lb.)*	28.67	30.97
Concentrates *(lb.)*	6.75	6.50
Total *(lb.)*	35.42	37.47
Feed dry matter consumed per 100 lb. of live wt. per day:		
Silage *(lb.)*	2.34	2.52
Concentrates *(lb.)*	0.55	0.53
Total *(lb.)*	2.89	3.05
Feed dry matter consumed per 100 lb. of FCM:		
Silage *(lb.)*	101.3	108.2
Concentrates *(lb.)*	23.9	22.7
Total *(lb.)*	125.2	130.9
Ratio, grain to milk	1:3.79	1:3.90
Av. vitamin A potency of milk per lb. butterfat:		
Initial *(I.U.)*	12,963.9	14,474.4
Final *(I.U.)*	10,718.6	9,151.4
Av. 30-day decline in potency *(I.U.)*	2,245.3	5,323.0

10 ft. × 25 ft. conventional concrete stave silo, and to a depth of 20 ft. in a 14 ft. × 40 ft. glass-lined, gas-tight steel silo. Filling procedures were the same as for Experiment I. The forage averaged 37% dry matter as stored in both silos.

The forage was weighed, sampled, and analyzed when stored and as removed and fed. Losses of dry matter and its constituents were calculated for both silos (Table 3). Temperatures during storage were recorded by means of thermocouples. Both silages, and a third silage (treated with SO_2), were fed to the limit of appetite as the sole roughage to six groups of three cows each during a 105-day double reversal, double Latin square design feeding trial. Grain consisting of six parts ground corn, four parts ground oats, 1% salt, and 1% bonemeal was apportioned at the beginning of each 35-day period at the rate of 1 lb. daily for each 4 lb. of fat corrected milk produced, and reduced by 2% every 7 days.

TABLE 3

Composition and storage losses of wilted alfalfa-grass silages stored in a conventional and in a gas-tight silo

Items	Dry matter	Constituents of dry matter							pH
		Protein[a]	Ether extract	Fiber	N-Free extract	Ash	Sugar	Carotene	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(γ/g.)	
Stored 25' deep in a 10' × 25' conventional silo (37% dry matter content)									
Chemical composition									
as stored	37.00	16.02	2.32	28.50	45.73	7.39	3.54	158.8	
as fed	36.07	16.14	2.97	30.60	42.36	7.93	0.89	52.6	4.36
Losses during storage (%)	12.53	11.88	+10.78	6.07	18.99	6.22	77.95	71.03	
Stored 20' deep in a 14' × 40' gas-tight silo (37% dry matter content)									
Chemical composition									
as stored	37.40	15.95	2.45	28.21	45.89	7.49	3.49	166.2	
as fed	35.17	16.43	2.98	32.02	40.73	7.83	.41	79.0	4.38
Losses during storage (%)	11.92	9.26	+7.17	0.04	21.82	8.07	89.69	58.13	

[a] Average of protein determined on wet samples and dry samples.

RESULTS

Dry matter and nutrient losses in the conventional silo were about the expected minimum for a 100-day storage period followed by a 4-month feeding period. Storage temperatures averaged somewhat lower than in the gas-tight silo (Table 3).

Dry matter and nutrient losses in the gas-tight silo were higher than expected, nearly approaching those in the conventional silo. This was probably due to air leakage, which caused a considerable rise in temperature in the silage remaining in the silo late in the feeding period, together with a small amount of spoiled silage. A subsequent check of the empty silo revealed air leakage in the unloader housing and through the pressure relief valve, which was fouled with rust and chaff.

Both silages had a mild pleasant odor, a low pH, and a relatively low ammonia nitrogen content. Sugar and N-free extract utilization during storage was a little higher in the gas-tight silo than in the conventional silo, perhaps because of air leakage. Despite the air leakage, carotene losses were considerably lower and the carotene content of the silage considerably higher in the gas-tight silo than in the conventional silo.

The results of the feeding trial (Table 4) show that both silages were very palatable and about equally so when fed to the limit of appetite. Feed dry matter and nutrient consumption were about equal. On both rations the cows received a considerable excess of digestible crude protein and 9% more total digestible nutrients than called for by the Morrison feeding standard.

Live weight changes, milk yield, and decline were about the same on both lots of silage. Thirty-day decline in milk production was about the same as for

TABLE 4

Results of the feeding trial with wilted silages stored in a conventional silo and a gas-tight silo

Items compared	Conventional silo	Gas-tight silo
Live wt. of cows:		
Initial wt. per cow *(lb.)*	1,129.5	1,129.6
Av. wt. per cow *(lb.)*	1,130.4	1,129.5
Av. gain or loss per cow per day *(lb.)*	+0.05	+0.03
Milk production (FCM):		
Initial per cow *(lb.)*	36.82	36.74
Av. per cow per day *(lb.)*	34.13	33.94
30-day decline *(lb.)*	4.58	4.44
30-day decline *(%)*	12.44	12.08
Feed dry matter consumed per cow per day:		
Silage *(lb.)*	25.73	25.54
Concentrates *(lb.)*	7.64	7.61
Total *(lb.)*	33.37	33.15
Feed dry matter consumed per 100 lb. of live wt. per day:		
Silage *(lb.)*	2.276	2.261
Concentrates *(lb.)*	0.676	0.674
Total *(lb.)*	2.952	2.935
Feed dry matter consumed per 100 lb. of FCM:		
Silage *(lb.)*	75.39	75.25
Concentrates *(lb.)*	22.38	22.42
Total *(lb.)*	97.77	97.67
Ratio, grain to milk	1:3.97	1:3.96

slightly wilted silage in Experiment I. The vitamin A potency of the milk produced was not determined.

Experiment III. A comparison of soybean silages stored in experimental 4 ft. × 8 ft. glass-lined, steel conventional, and gas-tight silos.

EXPERIMENTAL PROCEDURES

Heavy-yielding fine-stemmed soybeans, pods and seeds only partially formed, were harvested during a dry sunny spell in late August. They were cut with a ⅜-in. cutter setting, stored fresh green with 10% ground corn and cob meal added, also as wilted silage with nothing added, in both conventional and in gas-tight silos.

Both conventional and gas-tight silos (A) were tramped while being filled, filled to within 18 in. of the top, covered with Sisal Craft paper, filled to within 6 in. of the top, the whole covered with Sisal Craft paper and a steel follower weighted down with 1,000 lb. of bricks. One additional gas-tight silo (B) filled with wilted silage was filled without distributing tramping, division paper, or weighting to simulate filling methods in the farm-sized gas-tight silo.

The gas-tight silos (A) were sealed at the top with heavy Vinyl plastic fabric, taped against the outside of each silo near the top into a layer of soft rubber, producing a gas-tight seal. Some fullness was left in this fabric to allow for gas formation. The gas-tight silo (B) was sealed with a rigid steel cover imbedded in soft rubber. Both A and B silos were fitted with tubing to collect silage gases in an alkali solution without permitting air to enter the silo.

The silos were located inside a well ventilated, unheated building. The forage was weighed, sampled, and analyzed when stored, and thermocouples were installed for recording storage temperatures. Dry matter and nutrient losses were determined after 6 months storage, as shown in Table 5.

TABLE 5

Composition and storage losses of soybean silages stored in 4' × 8' experimental glass-lined steel conventional and gas-tight silos

Items and silo	Dry matter	Constituents of dry matter							
		Protein	Ether extract	Fiber	N-Free extract	Ash	Sugar	Carotene	pH
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(γ/g.)	
Fresh green + 10% corn and cob meal									
Chemical composition									
Crop as stored	32.54	16.66	2.24	22.74	47.35	11.02	2.56	177	
Silages									
Conventional	30.10	16.34	2.83	23.45	44.98	12.40	0.09	54	4.08
Gas-tight A	30.34	16.69	2.55	24.71	44.97	11.08	0.18	[a]	3.77
Losses below paper									
Conventional	8.23	9.99	+15.93	5.36	12.82	+3.26	96.79	71.77	
Gas-tight A	7.64	7.42	+5.16	+0.45	12.34	7.05	93.50	[a]	
Total losses for silo									
Conventional	17.80	19.44	+3.87	14.73	21.91	7.51	97.11	74.72	
Gas-tight A	8.13	9.69	+2.54	2.02	12.79	7.53	93.52	[a]	
Wilted — nothing added									
Chemical composition									
Crop as stored	45.40	18.90	1.90	27.47	42.45	9.28	3.16	212	
Silages									
Conventional	41.93	18.78	2.08	29.66	39.48	10.00	0.31	60	4.47
Gas-tight A	41.33	19.24	2.31	29.55	39.43	9.49	0.25	[a]	4.23
Gas-tight B	42.44	19.08	2.23	29.42	39.84	9.43	0.38	[a]	4.41
Losses below paper [b]									
Conventional	9.50	10.07	.90	2.28	15.83	2.48	91.14	74.19	
Gas-tight A	9.84	8.22	+9.61	3.01	16.24	7.99	92.85	[a]	
Total losses for silo									
Conventional	17.28	17.80	9.45	10.68	23.07	10.86	91.90	76.41	
Gas-tight A	9.49	7.87	+10.03	2.64	15.93	7.63	92.83	[a]	
Gas-tight B	8.65	7.78	+7.25	2.16	14.26	7.18	89.00	[a]	

[a] Carotene analyses of silages from gas-tight silos not run.

[b] No paper in gas-tight silo B.

RESULTS

There were only slight differences between the conventional silos and the corresponding gas-tight silos in storage temperatures or in chemical composition of the silages. All the silages had a very mild, pleasant odor and a low ammonia nitrogen content (7 to 8% of total). In every case, most of the available sugar was used up in the fermentation process. The gas-tight (A) silages were slightly lower in pH than the corresponding conventional silage. However, the gas-tight (B) untramped and unweighted silage was the same as its tramped and weighted conventional silo check.

Total dry matter losses ranged from 8.1 to 9.5% in the gas-tight silos, compared to 17.3 to 17.8% in the conventional silos. However, losses below the paper were about the same in both the conventional and gas-tight silos. The top spoilage which occurred in the conventional silos and not in the gas-tight silos represented nearly all the difference in preservation between the two types of silos.

DISCUSSION

The absence of top spoilage in the silages from sealed silos in Experiments I and III demonstrated that the extent of top spoilage and the nutrient losses therein are directly related to the amount of oxygen allowed to contact the stored forage. In the conventional silo, contact with oxygen causes top spoilage, which will be reduced if silo walls are tight and the silage is kept tightly packed against the silo wall during the settling period. Differences in preservation efficiency between the two types of silos will be small if the above precautions are observed.

The average farmer, however, too often neglects this necessary chore, the result being excessive top spoilage and side spoilage extending far down the silo wall. Thus, the advantage of the gas-tight silo could be as much as 10-15% in dry matter preserved, if proper attention is not given to compaction in a comparable conventional silo. In addition to the loss of many tons of feed, there are the losses of time and materials in storing the forage that must later be laboriously removed. It would appear that the time and cost of keeping the top properly tramped during the settling period is a wise investment in the conventional silo.

The results of Experiment I demonstrate that under anaerobic conditions less fermentation takes place in half-dry forage than in slightly wilted forage. This was evidenced by smaller losses of dry matter, nitrogen-free extract and sugar, and a higher pH. The larger field losses of dry matter and carotene of the half-dry forage more than offset the advantage of a smaller fermentation loss. This same experiment also demonstrated the flexibility of gas-tight silos as regards partial filling on widely separated dates without the development of top spoilage.

The development of an air leak in a gas-tight silo will, of course, eliminate much or all of its preservation advantages over a conventional silo. This was shown in Experiment II when the gas-tight silo developed an air leak and

exhibited a preservation efficiency about equal to that occurring in a conventional silo. This type of silo is equipped with a Vinyl plastic breather bag and a relief valve to compensate for gas pressure differentials between the inside and outside of the silo. The breather bag should be checked for flaws and holes before installation and after each filling. The relief valve may be fouled by dirt or forage particles and should, therefore, be protected during filling and its performance checked after a filling or partial filling has been completed. The filling hatch should be closed shortly after the silo is filled, first brushing the seating surfaces clean, then engaging the fastening dogs, and pulling the handles as tight as possible to provide an air-tight seal. The unloading hatch also should be kept closed when not in actual use; otherwise carbon dioxide may be forced out in the daytime as a result of the expansion of gas, and air can enter the silo through suction at night when the inside gas has contracted.

The high efficiency of preservation observed in Experiments I and III showed that distributing and tramping are not necessary when filling the gas-tight silo. However, some mechanical means of keeping the chopped forage nearly level in the silo throughout filling is desirable.

The automatic bottom unloader functions best when the forage is finely chopped and not too high in moisture. For this reason the gas-tight silo is particularly well adapted for storing slightly wilted or dryer forage, although fresh green forage can be handled successfully. Extra high moisture crops provide tremendous pressures and might sometime cause seepage and an air leak along a seam.

CONCLUSIONS

Silages stored in gas-tight silos and in conventional tower silos are similar in composition, palatability, aroma, quality, and feeding value. More dry matter, feed nutrients, and carotene are preserved in a gas-tight silo than in a conventional silo. The carotene content of the silage in the gas-tight silo was considerably higher. Below the top spoilage in the conventional silo, dry matter and nutrient losses were of about the same order as in the gas-tight silo. Differences in quantity and percentage preservation between the two types of silos can be stated as the quantity and percentage of the top and side spoilage losses in the conventional silo. Suitable operating precautions must be taken to keep the gas-tight silo from developing air leaks during the storage and feeding periods. Under anaerobic conditions, less fermentation takes place in half-dry forage than in slightly wilted forage. The gas-tight silo will allow greater operational freedom when dry matter content of forage, filling dates, and rate of silage feeding are considered.

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FURTHER STUDIES OF AUREOMYCIN IN FEED FORMULAS FOR DAIRY CALVES[1]

C. B. KNODT, E. B. ROSS, AND J. F. STEIN
Department of Dairy Husbandry,
The Pennsylvania Agricultural Experiment Station, State College

Some of the antibiotics have been found to be of value in increasing growth rates and decreasing the incidence of scours in young dairy calves. Aureomycin has been shown to have these beneficial effects (1, 2, 3, 5, 9, 11, 12, 13, 14, 15, 16, 17). Reports indicate that the feeding of terramycin to young dairy calves may also increase growth rate (4, 6, 10, 18). Potassium penicillin (7) and procaine penicillin (8) apparently do not increase growth rates at the levels studied.

Although several levels of aureomycin feeding have been studied in young dairy calves, the optimum does not appear to have been established. The objective of the studies presented in this report was to determine the effects of feeding higher levels of aureomycin to young dairy calves than those previously investigated at this station.

EXPERIMENTAL PROCEDURE

Four groups of 12 Holstein bull calves each, which were similar in body weight and withers height at 6 days of age, when they were placed on the experiment, were used in this trial. The calves were kept in individual pens and randomized so as to eliminate position as a variable. The pens were solid-walled, and each calf was provided with free access to water, hay, calf starter, and a salt block. Weekly measurements of body weight and withers height were taken of each calf on the same day of the week and at the same time of day. Daily observations were made of the condition of the feces. When scours persisted more than 24 hours, an 8-g. dose of sulfathalidine was administered.

The control group (I) was fed the following milk replacement formula: 50 lb. nonfat milk solids; 10 lb. dried whey; 15 lb. distillers' dried corn solubles; 10 lb. soluble blood flour; 7 lb. dextrose; 5 lb. oat flour; 0.5 lb. vitamin A and D concentrate (4,000 and 500 U.S.P. units per gram, respectively); 0.5 lb. trace mineral supplement, and 2 lb. dicalcium phosphate. During the milk replacement feeding period, the control replacement was supplemented with Aurofac 2 A (4 g. aureomycin per pound) at the expense of the nonfat milk solids as follows: Group II, 0.5%; Group III, 1.5%; Group IV, 2.5%. This resulted in levels of 2, 6, and 10 g. of aureomycin per 100 lb. of milk replacements. The formulas were mixed with water at 100° F. and fed in gruel form from open pails as follows: 1st-5th day, dam's milk; 6th-10th day, 2 lb. whole milk and 0.2 lb. milk replacement (twice daily); 11th-28th day, 0.5 lb.

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milk replacement and 5 lb. water (twice daily); 29th-42nd day, 0.6 lb. milk replacement and 6 lb. water (twice daily); 43rd-49th day, 0.7 lb. milk replacement and 7 lb. water (twice daily).

All groups of calves were fed ad libitum a good quality timothy-alfalfa hay during the trial. Calf starter was fed ad libitum up to a maximum of 6 lb. daily. The calf starter was formulated as follows: 416 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (44% protein-solvent process), 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. nonfat milk solids, 100 lb. distillers' dried corn solubles, 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt, and 4 lb. vitamin A and D_2 meal (1,814,544 and 226,800 U.S.P. units per pound, respectively.)

EXPERIMENTAL RESULTS

Data relative to the growth rates expressed as gains in body weights and withers heights of the groups of calves used in this trial are presented in Table 1. It can be noted that during the first 4 weeks the calves that received aureomycin gained body weight at a greater mean daily rate, which was statistically signifi-

TABLE 1

Effect of feeding aureomycin upon the growth rates of Holstein bull calves (12 calves per group)

Group	Aureomycin per 100 lb. milk replacement	Daily gain in body weight		Gain in withers height per period	
		0-4 wk.	0-7 wk.	0-4 wk.	0-7 wk.
	(g.)	*(lb.)*	*(lb.)*	*(in.)*	*(in.)*
I	0	0.93	1.28	1.31	2.69
II	2	1.21	1.54	1.83	3.25
III	6	1.23	1.45	1.54	2.95
IV	10	1.26	1.48	1.70	3.25

cant ($P < 0.05$), than did their controls. The differences in mean rates of gain in body weight between the groups that received aureomycin at the three levels studied were not significant during the first 4 weeks. A comparison of mean rates of gain in body weight during the 7 weeks of feeding also is presented in Table 1. The mean daily body weight gain of the aureomycin-fed groups during this period was at a significantly greater rate than the controls ($P < 0.05$). No statistically significant differences in growth rates in terms of body weight were found between the three groups receiving aureomycin at levels of 2, 6, and 10 g. per 100 lb. of milk replacement during the 7 weeks feeding period.

The rates of growth in terms of withers height also are presented in Table 1. Although the mean gains in withers height were greater for the aureomycin-fed groups, these differences only approached significance statistically ($P < 0.05$). Similarly, the differences in mean gain in withers height between the three groups that received aureomycin only approached statistical significance ($P < 0.05$).

TABLE 2

Hay and starter consumption of Holstein bull calves fed aureomycin supplemented milk replacements (12 calves per group)

Group	Aureomycin per 100 lb. milk replacement	Feed consumption		
		Starter		Hay
		Total per calf 0-7 wk.	per lb. of gain	Total per calf 0-7 wk.
	(g.)	*(lb.)*	*(lb.)*	*(lb.)*
I	0	109.5	1.55	26.5
II	2	120.5	1.51	29.5
III	6	118.0	1.50	29.0
IV	10	109.0	1.40	32.5

The consumption of hay and starter is presented in Table 2. Essentially no difference was found between groups in the efficiency of starter utilization on the basis of pounds of starter used per pound gain in body weight. Although the consumption of hay varied between groups during the 7 weeks feeding trial, the amount of hay consumed varied directly with the efficiency of starter consumed. Scours was not a problem, nor was its limited incidence related to any group. The feeding of aureomycin did not, therefore, influence the condition of the feces at any time in this trial.

SUMMARY

The feeding of 2, 6, and 10 g. of aureomycin per 100 lb. of milk replacement fed to Holstein bull calves resulted in statistically significant increased rates of gain in body weight as compared to gains of controls. Although mean gains in wither heights of all groups of calves were greater for those that received aureomycin during the 7 weeks trial, these differences only approached significance ($P < 0.05$).

No differences in efficiency of starter or hay utilization were found. The incidence of scours was low and not related to the aureomycin fed.

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METABOLIC FATE OF S^{35} IN THE LACTATING COW WHEN FED $S^{35}O_2$ PRESERVED SILAGE[1]

H. A. KEENER AND A. E. TEERI

Department of Dairy Husbandry,
New Hampshire Agricultural Experiment Station, Durham

AND

R. V. HARRINGTON[2] AND R. R. BALDWIN

Central Laboratories, General Foods Corporation, Hoboken, N. J.

The making of grass silage is becoming increasingly important as a method for preserving forage for the feeding of livestock. For this reason, a continuous search is being made for more efficient silage preservatives. One promising result of this search is the use of sulfur dioxide for this purpose. Studies on the use of this material as a silage preservative by Knodt (10), Knodt *et al.* (11), Skaggs (13), and Skaggs and Knodt (14) indicate that the nutrients of silage treated with this chemical are exceptionally well preserved and that the silage is well accepted by cattle. These conclusions are supported by unpublished data from this station, as well as by the experience of scores of farmers who have used sulfur dioxide as a silage preservative the past 2 years.

Although no one has reported any deleterious effects from feeding sulfur dioxide preserved silage to livestock, little work has been carried out to determine if such effects are possible. If large amounts of silage preserved with sulfur dioxide are to be fed to dairy cattle over long periods of time, it is important that a study on this subject be carried out. The first phase of such a study was an experiment to determine the metabolic fate of sulfur from sulfur dioxide used as a silage preservative. The results of this experiment are reported here.

EXPERIMENTAL

Preparation of radioactive SO_2. The radioactive SO_2 used in this experiment was prepared from elemental S^{35} obtained from the Oak Ridge National Laboratories of the U. S. Atomic Energy Commission, Oak Ridge, Tenn. S^{35} has a half life of 87.1 days and emits a beta particle of 0.167 m.e.v. A total of 30 mc. was used. The sulfur was oxidized quantitatively to SO_2, which was stored in three standard 550-ml. SO_2 gas tanks in amounts of 45.4, 90.8, and 136 g.

Preservation of silage. The silage was preserved in three 55-gal. steel drums. A large hole cut in the head of each drum was fitted with a gas-tight lid which

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[1] Scientific contribution No. 157 of the New Hampshire Agricultural Experiment Station. The radioactive SO_2 was made and the activity measurements were carried out at Central Laboratories of General Foods Corporation. The silage making and animal phases of the experiment including processing silage and excreta for counting were conducted at the New Hampshire Agricultural Experiment Station.

[2] Now at University of Colorado, Boulder.

could be cemented and bolted into place. The drums were coated on the inside with a Bakelite resin varnish. The forage used was a second cutting mixture of ladino clover, alsike clover, and a small percentage of timothy, and was mowed on July 11, 1951. It was field chopped without appreciable wilting and contained 79.2% moisture. The procedure used in making the silage was to place 100 lb. of the chopped forage in each drum. After the forage was compacted thoroughly by tramping, it was covered with two layers of sisal reinforced paper, and weighted down with 300 lb. of small clean stones. The lid was then secured.

Each lid was fitted with three one-hole rubber stoppers. The one nearest the outside was connected to an absorber train consisting of three flasks containing saturated $Ba(OH)_2$ solution and ending with a drying tube containing Ascarite. A second stopper was used for a thermocouple wire which extended to the center of the silage. A brass tube extending to the center of the forage was placed through the third stopper in the center of the lid. This was connected with one of the SO_2 cylinders. The number of hose connections was held to a minimum, and all were wired. All joints involving the rubber stoppers in the lid were treated with Glyptal. After the connections had been thoroughly inspected, all of the $S^{35}O_2$ in each of the three cylinders was allowed to flow into the forage in the three drums, so as to give three levels of SO_2 treatment. After this was done, the hose connections were closed with pinchcocks and the cylinders were removed. A suitable mask was worn by each person present as a protection against inhalation of the radioactive SO_2 in case of leakage, but there was no apparent loss of gas.

The drums were stored so that they were surrounded on the sides and covered with 10 to 12 in. of sawdust to prevent excessive loss of heat from the fermentation which took place. These conditions of storage prevailed until the silages were opened for feeding on December 9, 1951. Before the drums were opened, they were exhausted with the gas being passed through a saturated $Ba(OH)_2$ solution to collect any free $S^{35}O_2$ which might remain. Both the precipitates formed here and those collected during the storage period were almost devoid of sulfur. This indicated that the precipitates were primarily $BaCO_3$ and that essentially no SO_2 was lost from the silages. The silages were found to be of exceptionally good quality. Spoilage was negligible except for a small amount of silage which had become darkened where the coating came off of the drums.

Metabolism experiment. Three lactating cows were used for the metabolism studies. The equipment and procedures used were as outlined by Hansard (8) and Hobbs *et al.* (9). Each cow was assigned to a particular silage and was given all she would eat of it until 36 kg. had been consumed. This took approximately 30 hours. During this period the cows were given 1 kg. of a concentrate mixture twice per day. After the radioactive silage had been consumed, the concentrate allowance was doubled. The roughage part of the ration consisted of grass silage for 5 days, after which it was changed to hay. All urine, feces, and milk were weighed and sampled. Blood samples were taken periodically.

Preparation of $BaSO_4$ samples for assay. The individual and composite samples prepared for assay are indicated in the section on results and discussion.

Total sulfur was determined on all of these samples in order that both rates of excretion and balances could be determined. In addition, composite samples of urine for the first 4.5 days also were analyzed for total sulfate and inorganic sulfate. Samples of protein were obtained from the composite samples of milk covering this same period. After purification, these samples of milk protein were analyzed for total sulfur. The silages were analyzed for total sulfur, and some data were obtained on the chemical state of the sulfur. Direct activity measurements were made on the blood and on several blood fractions.

Total sulfur determinations were carried out according to Givens' modification (7) of Benedict's method (1), and total sulfate and inorganic sulfate were measured by the method of Folin (6). These gravimetric methods were modified slightly in that relatively large samples and correspondingly greater amounts of the various reagents were employed in order to obtain sufficient barium sulfate for radioactivity measurements. In the total sulfur determinations an excess of oxidizing mixture was used and the heating, of sufficient duration, was carried out with utmost care in order to insure complete oxidation of the sample and decomposition of the remaining reagent. Because the samples were so large (100 ml. for milk and urine, 50 g. for feces and silage), this oxidation involved heating first on the steam bath over night, or longer, and then on the electric hotplate for completion of the determination by the usual procedure.

Proteins were separated from milk by precipitation with trichloracetic acid and extraction with hot acetone and ether as described by Block *et al.* (4).

Assay of samples. Ten to 80-mg. samples of the $BaSO_4$ from the urine, feces, milk, and silage were weighed in a sintered glass filter calibrated for use with the Berstein-Ballentine methane flow counter (2). The $BaSO_4$ was ground and slurried with water, and suction was applied to form a mat. The precipitate was washed on the central sintered glass section with ethanol and dried. The filter then was placed on the counting tube and a count was taken. In order to obtain the specific activity, this was converted into counts per minute, corrected for radioactive decay to January 1, 1952, divided by the weight of the $BaSO_4$ aliquot, and corrected for self-absorption with previously calculated absorption curves. To obtain a value for the total activity in the sample, the specific activity in terms of counts per minute per milligram was multiplied by the total milligrams of $BaSO_4$ in the sample.

Where the activity was too high to be measured accurately with the scalers, the samples were diluted by a factor of ten with nonradioactive $BaSO_4$, and an aliquot of the diluted sample was assayed for radioactivity.

For the blood, 5 ml. of the citrated whole sample was evaporated to dryness in a 4.5-cm. dish in a current of warm air. The samples were counted at a constant geometry with an end window tube.

In order to determine the distribution of radioactivity, 10-ml. samples of citrated whole blood were centrifuged for 10 minutes at 6,400 r.p.m., and the cells were washed twice with isotonic saline. Then 2 ml. of 10% trichloracetic acid was added to the plasma and the protein fraction was separated. All of the fractions were dried in 4.5-cm. dishes and counted with the end window tube.

In order to measure the change in sulfur distribution with time, a blood sample from each cow was fractionated at 24 hours and at 6.5 or 9.5 days. The total activity was taken as the sum of the activities in the various fractions. Because self absorption corrections were not applied, these figures are not absolute but serve to give a relative picture of the distribution. As with the other activity measurements, all blood counts were corrected to January 1, 1952.

RESULTS AND DISCUSSION

Data on the amount of $S^{35}O_2$ used in each of the three silages, the chemical state of the total sulfur at the time of feeding, and the metabolic fate of the S^{35} when the silages were fed to dairy cows are given in Table 1. Information on the rates of excretion of S^{35} in the urine, feces, and milk is given in Figure 1, and information on periodic S^{35} levels in the blood is given in Figure 2.

These results show that practically all of the S^{35} added to the silage in the form of $S^{35}O_2$ remained in a water soluble state. This explains why at least two-thirds of the S^{35} from the silages was absorbed from the digestive tract and then

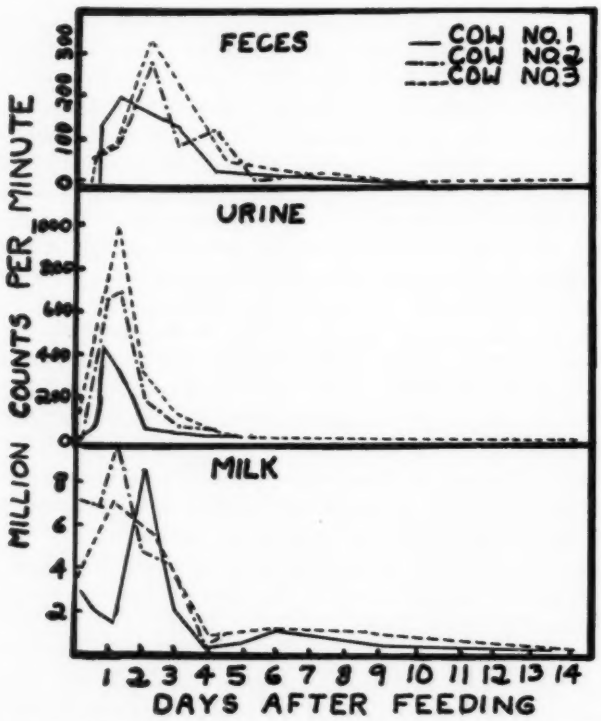

FIG. 1. Total S^{35} activity excreted in feces, urine, and milk during various periods following initial $S^{35}O_2$ silage feeding.

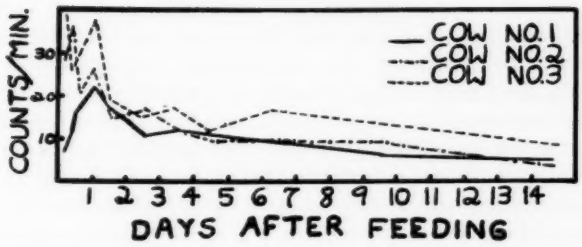

FIG. 2. Relative S^{35} activities of blood taken periodically after initial $S^{35}O_2$ silage feeding.

eliminated by the kidneys. It was not possible to determine how much more sulfur was absorbed and then excreted through the bile or passed into the digestive tract by other means. Most of the remaining S^{35} consumed was eliminated in the feces, and only 1 to 2% of it was eliminated in the milk. In considering the physiological problem involved, it should be kept in mind that the silage containing the S^{35} was consumed over a period of approximately 30 hours and that none was fed thereafter. Four hours after feeding started, there were appreciable amounts of S^{35} in the blood. Eight hours after feeding started, relatively large amounts were in the urine, and there were small amounts in the feces. Considerable activity was found in the milk at the first milking (15 hours) after feeding started. By 54 hours after the last $S^{35}O_2$ silage was eaten, urinary and mammary excretion of S^{35} had decreased markedly, and the amount excreted after that time was a relatively small part of the total. As might be expected, there was some lag as far as fecal excretion of the S^{35} was concerned. Two to three more days were required for the elimination of most of the activity from the digestive tract. These results indicate that a major portion of the sulfur added to silage in the form of SO_2 is absorbed from the digestive tract and then rapidly eliminated by the kidneys.

TABLE 1

Summary of distribution of sulfur in $S^{35}O_2$ preserved silage and its metabolic fate when fed to dairy cows

	Original forage	Silage fed to cow No. 1	Silage fed to cow No. 2	Silage fed to cow No. 3
Rate $S^{35}O_2$ used *(lb./T.)*	.....	2	4	6
Chemical state of total S in silage *(d.b.)*				
Sulfur dioxide *(%)*	0.00	0.05	0.19	0.55
Water sol. sulfate *(%)*	0.62	0.93	1.27	1.70
Water insol. sulfate *(%)*	0.39	0.45	0.49	0.57
S^{35} activity fed *(million cpm)*		1330.6	2587.4	4071.5
S^{35} activity recovered				
Feces *(%)*		43.8	26.5	24.9
Urine *(%)*		69.8	70.9	67.5
Milk *(%)*		1.8	1.7	1.0
Total *(%)*		115.4	99.1	93.4

The next consideration is whether the elimination of the amounts of sulfur involved puts any strain on the kidneys. If a cow eats 75 lb. per day of a silage preserved with 6 lb. of SO_2 per ton, the kidneys would need to eliminate approximately 34 g. of sulfur from SO_2 per day. Besides the amount of sulfur that must be eliminated, the chemical state in which it is eliminated also must be considered. It was found that on the average approximately 87% of the total urinary S^{35} was in the oxidized form and that on the average 83% of the oxidized S^{35} was in the inorganic form. According to Denis and Hobson (5), the sulfate ion is excreted with greater difficulty than any other inorganic radical ordinarily present in normal blood. A review of the literature revealed nothing else which would indicate any possible harmful effects from the absorption or elimination of this amount of sulfur. Although there probably is little likelihood that the excretion of relatively large amounts of oxidized sulfur from SO_2 silage might cause renal damage, it does seem that studies on this problem should be undertaken.

The nutritive value of the sulfur obtained from SO_2 used as a silage preservative seems to be of some practical importance. This may be particularly true where concentrates containing urea are fed with low protein roughages. It was found that between 6.5 and 9.5 days after the first silage was fed, 40 to 50% of the total activity in the blood was in the plasma protein, while the maximum in the protein free plasma had decreased to about 12%. At this same time the blood cells contained a maximum of about 10% of the total activity and the cell wash about 30 to 50%.

Studies on the milk protein isolated from composite samples saved during the first 4.5 days after silage feeding started showed that sulfur from the SO_2 also was synthesized into milk proteins. It was found that approximately 10 to 12% of the total activity in the milk was in the protein fraction. The specific activity of the milk protein increased with each increase in activity of the silage fed, but at a slower rate. There was little change in the sulfate content of the milk protein, however.

These results on the nutritive value of sulfur obtained from SO_2 are in agreement with those reported by other workers. Thomas *et al.* (15) reported that lambs have a dietary requirement for sulfur and that it can be met by feeding inorganic sulfates. In the absence of dietary sulfur, urea nitrogen apparently was not utilized. Block *et al.* (4) found that when $Na_2S^{35}O_4$ was fed to a goat, activity appeared in the milk within 3 hours. The radioactive sulfur was found in both methionine and cystine when the milk proteins were hydrolyzed and the amino acids fractionated. When this compound was fed to a ewe, the greater part of the activity of the rumen contents was found in the precipitate formed by trichloracetic acid. Appreciable amounts of activity were found in the cystine and methionine isolated from this precipitate. Block and Stekol (3) found that when $Na_2S^{35}O_4$ was fed to a cow, the milk proteins also showed appreciable radioactivity. This likewise was true for the methionine and cystine separated from the milk proteins.

SUMMARY AND CONCLUSIONS

The metabolic fate of sulfur from $S^{35}O_2$ used as a silage preservative was studied with lactating cows. Approximately two-thirds of the S^{35} fed was absorbed and then for the most part eliminated by the kidneys. It required approximately 54 hours after the last $S^{35}O_2$ silage was consumed to eliminate most of the activity from the urine and the milk, but there was a lag of approximately 2 days in eliminating the S^{35} from the feces. The fact that sulfur from the $S^{35}O_2$ was synthesized into the proteins of the blood and of the milk indicates that it may be of value from a nutritional standpoint.

These results suggest the desirability of determining any effects on the kidneys from feeding relatively large amounts of inorganic sulfates to dairy cattle.

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CHARACTERISTICS OF AN ENDOCELLULAR PROTEOLYTIC ENZYME SYSTEM OF *STREPTOCOCCUS LACTIS*[1]

W. C. VAN DER ZANT AND F. E. NELSON
Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

In a previous paper (9) it was reported that *Streptococcus lactis* growing in milk caused a rapid increase in soluble nitrogen and also in tyrosine and tryptophan during the first 24 hours, followed by a smaller but gradual increase during the rest of the experimental period. Attempts to demonstrate the proteolytic system in the cell-free culture medium after organism growth were not successful. Amundstad (2) reported that a cell-free extract of two strains of *S. lactis* grown in a broth medium showed optimum activity at pH 6.6 to 6.8 on both peptone broth and caseinate solutions, as determined by the increase in acid-soluble nitrogen and amino nitrogen. A cell-free extract of *S. cremoris* showed optimum activity at pH 6.3 to 6.5 and proved to have greater proteolytic activity than the extract prepared from *S. lactis*. Baribo and Foster (3) determined some of the characteristics of a cell-free extract of one strain of *S. lactis* grown in carrot-liver broth.

In the present investigation cell-free extracts were prepared by sonic vibration from cells of *S. lactis* grown in skimmilk and several broth media and were tested for proteolytic activity against skimmilk, casein, and lactalbumin.

EXPERIMENTAL METHODS

The two cultures of *S. lactis* used were strains 18 and 26 used in the previous study (9); they were handled as described in that publication. Cultures were transferred daily for three transfers prior to each trial, using the culture medium employed in the experiment. One per cent inoculum was used in all cases, with incubation at 32° C.

Preparation of cell-free extracts. In most of the experiments *S. lactis* was grown in vitamin-test casein medium B and also in skimmilk, which were dispensed in 6-l. quantities in Pyrex carboys. The milk was heated at 85° C. for 20 minutes, rapidly cooled to 32° C., adjusted to pH 6.8 to 7.0, and inoculated. Vitamin-test casein medium B contained 20 g. vitamin-test casein (Nutritional Biochemicals), 10 g. yeast extract, 6.5 g. glucose, 10 g. $K_2HPO_4 \cdot 3H_2O$, and 1,000 ml. distilled water. The casein medium was autoclaved for 13 minutes at 15 lb. pressure, cooled to 32° C., adjusted to pH 6.8 to 7.0 and inoculated. The inoculated media were incubated for 8 to 10 hours, an incubation period which gave a satisfactory yield of young, metabolically active cells of high proteolytic activity. During incubation the reaction was kept at pH 6.8 to 7.0 by addition of sodium hydroxide. The cells from the casein medium were collected in a

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Sharples supercentrifuge operated at 30,000 r.p.m. They were washed twice with ice-cold *M*/15 phosphate buffer (pH 7.0), recovered by centrifugation in a refrigerated centrifuge, and stored in a refrigerator.

After incubation, the milk was run through a Sharples supercentrifuge at 30,000 r.p.m. The material collected in the bowl of the centrifuge was cooled immediately to 2° C., mixed with a few grams of sterile sea sand and some sterile distilled water and ground in a chilled mortar until all material had gone into a fine suspension. Centrifugation at 4,000 r.p.m. for 30 minutes separated this material into distinct layers, the cells forming the top layer. The cells were removed with a spatula and washed and stored as described above.

In the early phases of this work cell-free extracts were prepared by subjecting *S. lactis* cells to alternate freezing and thawing and also by grinding the cells with alumina and ground Pyrex glass between concentric glass cones, as described by Utter and Werkman (8). However, more uniform extracts were prepared by sonic vibration. Cell-free extracts were prepared as follows: Seven ml. *M*/15 phosphate buffer (pH 7.0) were added to 7 g. of wet cells and stored overnight in a freezer unit. Next day 7 ml. *M*/15 phosphate buffer (pH 7.0) were added to the frozen suspension. After thawing, the suspension was poured into the chilled cup of a Raytheon 9 kc. Magnetostriction oscillator. Disintegration was allowed to take place for 90 minutes, during which the cup was cooled by circulating ice cold water. Disintegration for a 90-minute period produced extracts with greater proteolytic activity than when shorter or longer periods were used. Intact cells and cell debris were removed by centrifugation. The clear supernatant was removed with a medicine dropper. The debris was mixed with 7 ml. ice cold *M*/15 phosphate buffer (pH 7.0), and the supernatant collected after centrifugation was added to the first portion. The combined extracts were stored in a freezer unit and used within a few days.

Preparation and analysis of digestion mixture. Skimmilk and 2% solutions of casein and lactalbumin were used as substrates. Pasteurized skimmilk was brought to the desired pH and stored at 2° C. A 2% casein solution was prepared by suspending 4 g. of casein (Fischer Scientific Co.) in approximately 100 ml. distilled water, adjusting to pH 10.0, and heating in a steamer for 10 to 15 minutes. After cooling, the solution was adjusted to the desired pH, followed by addition of 20 ml. of a 0.2 *M* composite buffer of that pH value, and made up to volume with sterile distilled water. Two per cent solutions of α-, β- and γ-casein were prepared as described for casein. A 2% solution of lactalbumin (Supplee Research Co.) was prepared as described for casein. Merthiolate was added to all substrates as preservative at the rate of 1 mg. to 40 ml. substrate; this concentration had no adverse effect on the enzyme actions studied. All substrates were stored at 2° C.

The digestion mixture, in sterile screw-top test tubes, consisted of 5 ml. of substrate, plus cell-free extract and sterile distilled water to make a total volume of 7 ml. The cell-free extract was added when the substrate had reached the temperature of incubation. Unless stated otherwise, 1 ml. of cell-free extract was added to the substrate and incubated at pH 7.0 in a waterbath at 37.5° C.

for 18 hours. After incubation, tyrosine and tryptophan were determined in a trichloroacetic acid filtrate of milk prepared as described by Hull (5), except that 1 ml. of filtrate and 4 ml. of distilled water were used with the original concentration of acid. The blue color which appeared after Folin-Ciocalteau reagent (4) was added to the protein-free filtrate was measured in a Klett-Summerson photoelectric colorimeter using 645 mμ wave-length filter. The procedure followed for the casein and albumin substrates was similar to the one used for milk, except that 0.6 *N* trichloroacetic acid was used, whereas for milk 0.72 *N* acid was used. Previously prepared standard curves, showing the per cent light transmission for various tyrosine concentrations, then were used to convert the sample reading into its tyrosine equivalent. In calculating the results of this test, the tyrosine content is based upon its concentration in 1 ml. of filtrate used for the production of the blue color. Since the substrates and cell-free extracts used commonly contained some color-producing products, as shown by this test, initial values were obtained on them by adding the required amount of cell-free extract to the substrates after the trichloroacetic acid was added and then carrying out the remainder of the determination. The amount of tyrosine and tryptophan found in these 1-ml. quantities of protein-free filtrates ranged from 10 to 15 γ. Proteolysis was expressed as the increase in tyrosine and tryptophan per milliliter of filtrate above the values obtained for substrate on which enzyme had not acted.

Bacterial counts were made according to *Standard Methods for the Examination of Dairy Products* (1), with incubation at 32° C.

RESULTS

Characteristics of cell-free extract of S. lactis grown in milk. In Figures 1 and 2 are presented representative data showing the effect of pH, increasing quantities of cell-free extract, and time and temperature of incubation on the digestion of skimmilk, casein, and lactalbumin by a cell-free extract of *S. lactis* 26. Maximum activity against skimmilk and casein was found at pH 7.0, with some indication of a second optimum at pH 5.5 (Figure 1A). Optimum pH for lactalbumin was found at pH 6.5. As shown in Figure 1B, there appeared to be a direct relationship between the quantity of cell-free extract used and digestion of skimmilk, casein and lactalbumin, at least up to 1 ml. of cell-free extract. Up to 18 to 20 hours of incubation (Figure 2A), a direct relationship between the time of incubation and amount of digestion of all three substrates existed. Optimum activity against skimmilk, casein, and lactalbumin, within the temperature limits studied, was found at 45° C. (Figure 2B); however, the increase in rate with temperature was somewhat less above 37° C. The results found with a cell-free extract prepared from cells of *S. lactis* 18 were similar to those reported for strain 26.

To avoid difficulties encountered in separating *S. lactis* cells from milk, attempts were made to derive a medium for growing *S. lactis* cells from which extracts could be prepared with a proteolytic activity comparable to that of an

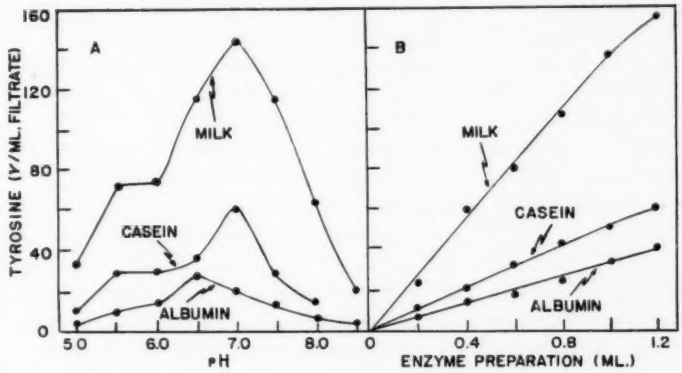

FIG. 1. Effect of pH and increasing quantities of enzyme preparation on the proteolysis (expressed as increase in tyrosine) of milk, casein, and albumin by enzyme preparation from strain 26 grown in milk

A: 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. for 18 hours
B: ml. enzyme preparation per 5 ml. substrate incubated at pH 7.0 and 37.5° C. for 18 hours

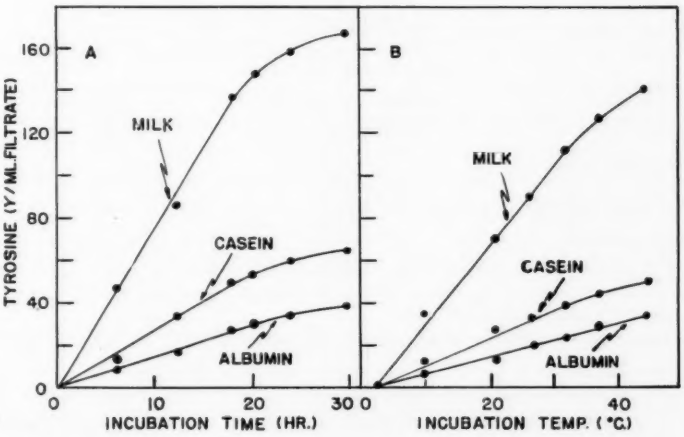

FIG. 2. Effect of time and temperature of incubation on the proteolysis (expressed as increase in tyrosine) of milk, casein, and albumin by enzyme preparation from strain 26 grown in milk

A: 1 ml. enzyme preparation per 5 ml. substrate incubated at pH 7.0 and 37.5° C.
B: 1 ml. enzyme preparation per 5 ml. substrate incubated at pH 7.0 for 18 hours

extract from cells grown in milk. *S. lactis* 26 was grown in the following media: *(a)* the simplified amino acid medium of Niven and Sherman (6); *(b)* a medium used by Amundstad (2) to grow *S. lactis* for the preparation of cell-free extracts, containing 20 g. peptone, 10 g. glucose, 10 g. lactose, 1 g. $MgSO_4$, 2 g. K_2HPO_4,

2 g. NaCl, 500 ml. yeast extract and 455 ml. water; *(c)* vitamin-test casein medium A containing 20 g. vitamin-test casein, 6.5 g. glucose, 10 g. $K_2HPO_4 \cdot 3H_2O$, 1 mg. riboflavin, 1 mg. calcium pantothenate, 1 mg. nicotinic acid, 1 mg. pyridoxine HCl, 1 mg. thiamine, 1 γ biotin, 10 γ folic acid, 20 γ vitamin B_{12} and 1000 ml. distilled water; *(d)* vitamin-test casein medium B, which had the same composition as *c* except that the vitamins were replaced by 10 g. yeast extract; *(e)* medium *d* except casein was replaced with an equal amount of tryptone; *(f)* medium *c* in which the vitamin-test casein was replaced with an equal amount of technical casein; *(g)* tryptone glucose meat extract broth (1). All media were autoclaved for 13 minutes at 15 lb. pressure, rapidly cooled to 32° C., adjusted to pH 6.8 to 7.0, and inoculated with 1% of a culture of *S. lactis* 26, which had been transferred daily for three transfers in the culture medium employed. Incubation at 32° C. for 10 hours gave a satisfactory yield of young, metabolically active cells. During incubation the reaction was kept at pH 6.8 to 7.0 by addition of sodium hydroxide. After incubation, the cells were harvested and cell-free extracts were prepared as described previously. One ml. of extract represented approximately 3×10^{10} cells in each case. The number of viable cells per milliliter of medium after incubation for 10 hours and the proteolytic activity of the extracts against skimmilk are presented in Table 1. Of all the

TABLE 1

Proteolytic activity of cell-free extracts[a] of S. lactis 26 grown in different media[b] (skimmilk test substratum)

Medium	Standard plate count	Tyrosine
	(millions per ml.)	*(γ per ml. filtrate)*
Niven and Sherman	570	29
Amundstad	1100	35
Vitamin-test casein A	1200	65
Vitamin-test casein B	2000	99
Tryptone medium	1800	42
Casein medium	1400	61
T.G.E. broth	900	38

[a] 1 ml. extract represents 3×10^{10} cells.

[b] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.

extracts, the one prepared from cells harvested from vitamin-test casein medium B showed a proteolytic activity closest to that of an extract prepared from *S. lactis* grown in milk. When casein was present in the growth medium, greater proteolytic activities were found in the cell-free preparations than when peptone or tryptone was used; the smallest activity was found when proteins or peptides were omitted from the medium. All growth media containing casein supported excellent growth, as shown in Table 1. These results seem to suggest that whole protein (casein) in the growth medium stimulated the proteolytic activity of the cell-free extract.

The possible effect of tryptic digestion of the casein in vitamin-test casein medium A on the proteolytic activity of the cell-free extract was investigated. Sterile 2% solutions of vitamin-test casein were adjusted to pH 8.0 and digested

TABLE 2

Effect of tryptic digestion of casein in medium on the proteolytic activity of the cell-free extract[a] of S. lactis 26[b] (skimmilk test substratum)

Medium	Standard plate count	Tyrosine
	(millions per ml.)	*(γ per ml. filtrate)*
No digestion	1900	60
Digestion for 10 hours	1800	25
Digestion for 20 hours	1800	25
Digestion for 40 hours	1700	22

[a] 1 ml. extract represents 3×10^{10} cells.
[b] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.

with 0.05% trypsin for periods of 10, 20, and 40 hours, followed by sterilization for 15 minutes at 15 lb. pressure. The other components of vitamin-test casein medium A then were added and the media inoculated with *S. lactis* 26 (1%), followed by incubation at 32° C. for 10 hours. Vitamin-test casein medium with undigested casein which received the same heat treatment as the media with digested casein was used as the control. Approximately the same number of viable cells (1800×10^6 per ml.) was harvested from the media containing digested and undigested casein. The cell-free extracts (1 ml. of cell-free extract represented approximately 3×10^{10} cells) prepared from cells grown in the media containing trypsin-digested casein showed less than 50% of the proteolytic activity of the extract prepared from cells grown in the medium containing undigested casein (Table 2).

A study of the effect of omission of individual vitamins from vitamin-test casein medium A on the proteolytic activity of the cell-free extract was made. In each case, 1 ml. of cell-free extract represented approximately 4×10^{10} cells. Except for nicotinic acid, omission of one vitamin did not affect appreciably the proteolytic activity of the cell-free extract (Table 3). Omission of individual vitamins from vitamin-test casein medium A affected the growth greatly. In order to verify that nicotinic acid was not merely acting as an activator for the enzyme system varying amounts of nicotinic acid up to 1 mg. were added to a

TABLE 3

Effect of omission of vitamins from vitamin-test casein medium A on the proteolytic activity of the cell-free extract[a] of S. lactis 26[b] (skimmilk test substratum)

Vitamin omitted	Standard plate count	Tyrosine
	(millions per ml.)	*(γ per ml. filtrate)*
All	0.1	—
Riboflavin	720	79
Ca-pantothenate	110	78
Nicotinic acid	170	59
Pyridoxine	200	76
Thiamine	710	79
Biotin	540	73
Folic acid	790	77
Vitamin B_{12}	870	78
None	1260	80

[a] 1 ml. extract represents 4×10^{10} cells.
[b] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.

digestion mixture consisting of milk and cell-free extract prepared from *S. lactis* grown in nicotinic acid-deficient vitamin-test casein medium A. No increase in proteolytic activity was found in these mixtures with added nicotinic acid.

Characteristics of cell-free extract of S. lactis 26 grown in vitamin-test casein medium B. Data on the effect of pH, increasing quantities of cell-free extract, and time and temperature of incubation on the digestion of skimmilk, casein, and lactalbumin indicated that except for the lack of an inflection in the curves at pH 5.5 to 6.0, the results on cells grown in vitamin-test casein medium B parallel closely those of cells grown in milk. Data on the effect of pH on the activity of the cell-free extract against 2% solutions of α-, β- and γ-casein are presented in Figure 3. A slightly greater hydrolysis of α-casein than of β- and

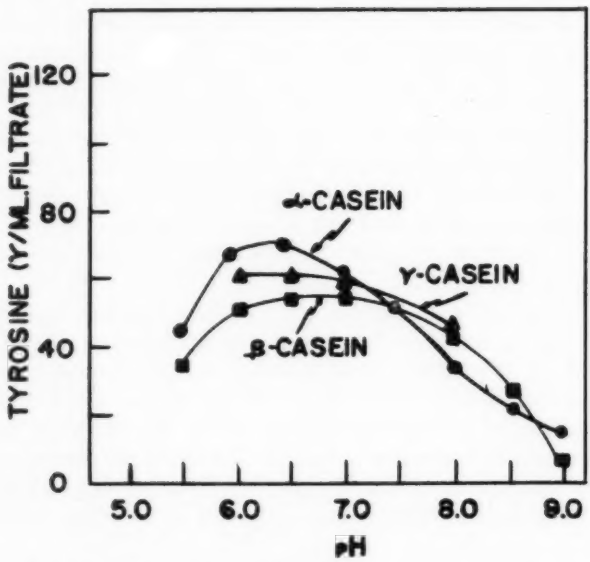

FIG. 3. Effect of pH on the proteolysis (expressed as increase in tyrosine) of α-, β-, and γ-casein by enzyme preparation from strain 26 grown in vitamin-test casein medium B (1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. for 18 hours)

γ-casein was observed at pH 6.0 to 7.0. Maximum activity against α-casein was found at pH 6.5, for β-casein at pH 6.5 to 7.0, and for γ-casein at pH 6.0 to 7.0.

Another study showed that the proteolysis found when 1 ml. of cell-free extract was incubated for 18 hours at 37.5° C. with 5 ml. of substrate containing casein and albumin in the proportions commonly found in milk was smaller than when 5 ml. of milk was used as substrate. After incubation, 1 ml. of protein-free filtrate of milk contained 132 γ of tyrosine and tryptophan, whereas the filtrate prepared from the solution containing casein and albumin contained 92 γ of tyrosine and tryptophan.

Effect of reducing agents on activity of cell-free extract. One-ml. quantities of various reducing agents in 0.1, 0.01, and 0.001 *M* concentration were incubated with 1 ml. of cell-free extract at 37.5° C. for 1 hour. After addition of substrate (skimmilk), the activity was determined after incubation at 37.5° C. and pH 7.0 for 18 hours. The data (Table 4) show that all reducing agents used in 0.1 and 0.01 *M* concentration increased the proteolytic activity of the extract, whereas a 0.001 *M* concentration, except for Na_2SO_3, did not show any appreciable activation. Na_2SO_3 proved to be the best activator.

TABLE 4

Effect of reducing agents on the proteolytic activity of a cell-free extract of S. lactis 26[a] *(skimmilk test substratum)*

Reducing agent	Tyrosine (γ per ml. filtrate) when reducing agent was added in concentration of:		
	0.1 *M*	0.01 *M*	0.001 *M*
None	96	96	98
Thioglycollate	140	118	100
Na_2SO_3	151	132	110
KCN	111	106	100
Ascorbic acid	116	102	100

[a] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.

Effect of metallic ions on activity of cell-free extract. One-ml. quantities of the following solutions in 0.1, 0.01 and 0.001 *M* concentrations were used: Ca $(C_3H_5O_3)_2 \cdot 5H_2O$, $Fe_2(SO_4)_3$, $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $CoSO_4 \cdot 7H_2O$. The cell-free preparations (1 ml.) were incubated with the metal solution for 1 hour at 37.5° C., followed by incubation with skimmilk at pH 7.0 and 37.5° C. for 18 hours. The ions either had essentially no effect (Ca^{++}) or were slightly inhibitory.

Stability of proteolytic activity of cell-free extract. Twenty-ml. portions of the extract were adjusted to pH values ranging from 5.0 to 9.0 with dilute sodium hydroxide or sulfuric acid and adjusted to the same volume (23 ml.). For each trial 3 ml. of extract were placed in a test tube and heated at 50, 55, and 61.7° C. for different lengths of time. After the heat treatment, the tube was plunged in

TABLE 5

Effect of heat at different pH levels on the proteolytic activity of a cell-free extract of S. lactis 26[a] *(skimmilk test substratum)*

pH of extract	Tyrosine (γ per ml. filtrate[b]) after heating enzyme at:							
	50° C.			55° C.			61.7° C.	
	5 min.	15 min.	25 min.	5 min.	10 min.	15 min.	1 min.	2 min.
5	34	30	24	18	6	0	0	0
6	62	48	38	30	18	6	0	0
7	78	64	58	40	27	10	5	0
8	68	42	40	28	12	0	0	0
9	36	34	26	14	10	0	0	0

[a] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.
[b] Unheated extract: 100γ tyrosine per ml. filtrate at pH 7.0.

ice water to cool the contents. One-ml. portions were used to test for proteolytic activity against skimmilk at pH 7.0 and to prepare the blank. Maximum stability of the activity (Table 5) at all three temperatures seemed to occur at pH 7.0. Heating at 61.7° C. for only 2 minutes inactivated the preparation adjusted to pH 7.0. The extract was inactivated quite rapidly at pH 6.0 and 9.0, even at the lower temperatures.

For determination of the stability of proteolytic activity when cell-free extracts are held at different pH levels, extracts were adjusted to pH values ranging from 5.0 to 9.0, as described previously, and stored at 2 and 32° C. for various lengths of time. The activity against skimmilk (Table 6) appeared to be quite stable between pH 5.0 and 9.0, with storage at 2° C. A considerable destruction was noted at pH 5.0 and 9.0 when stored at 32° C. and a somewhat smaller destruction at pH 6.0 and 8.0 at 32° C.

TABLE 6

Stability of proteolytic activity at different pH levels when held at 2 and 32° C. for various lengths of time[a] (skimmilk substratum)

Time (hours)	Tyrosine (γ per ml. filtrate[b]) after holding enzyme at pH:				
	5.0	6.0	7.0	8.0	9.0
			2° C.		
12	107	120	128	117	112
24	100	117	124	117	107
96	96	112	123	104	95
			32° C.		
12	46	102	129	98	30
24	40	83	123	102	26
96	32	80	112	83	20

[a] 1 ml. enzyme preparation per 5 ml. substrate incubated at 37.5° C. and pH 7.0 for 18 hr.
[b] Control = 128 γ tyrosine per ml. filtrate.

DISCUSSION

The endocellular proteolytic system of *S. lactis* appears to be partially adaptive, since replacement in the growth medium of whole protein (casein) with breakdown products of protein caused definite decreases of the proteolytic activity. The presence of nicotinic acid in the growth medium seems to be involved in some manner in the production rather than activation of the proteolytic activity. Continued growth and production of proteolytic activity after omission of one vitamin at a time may be due either to ability of the organism to synthesize these nutrients or to presence of these compounds in the vitamin-test casein used. Milk used as substrate demonstrated more proteolysis than the combined fractions of casein and albumin, which may have been due to *(a)* breakdown of one or more noncasein, nonalbumin nitrogen fractions of milk or *(b)* factors inherent in milk which promote the activity of the enzyme system. Furthermore, in an impure system such as this in which a multiplicity of enzymes may be present, peptidases may act on some of the smaller protein

fragments and be partially responsible for the increased hydrolysis. The effect of pH, temperature of incubation, metallic ions and reducing agents on the proteinase activity of a cell-free extract of *S. lactis* 26 was very similar to that reported for a different strain by Baribo and Foster (3), although most of the present study was made using pH 6.8-7.0 instead of pH 5.1 as employed by Baribo and Foster. However, the proteinase in the present study was much less stable against heat and acid reaction than the one described by Baribo and Foster.

S. lactis may be one of the sources of microbial proteinase important in cheese ripening. Extracts of cheddar cheese (3, 7) have been found to be most active at pH 5.0; a secondary optimum occurs near neutrality. The low level of proteolytic activity at pH 5.0 found in this study would limit the activity in cheese of most common types; the enzyme might be active on the surface of cheese such as limburger where the pH is raised.

In a previous study (9), however, it was found that a somewhat delayed but marked increase in soluble nitrogen occurred in a milk culture of *S. lactis* at pH 5.5. It may be that this component of the proteolytic activity has greater stability at the pH of ripening cheddar cheese than the component active at pH 7.0 and may be part of the proteolytic activity of cheese extracts with optimum at pH 5.0 to 5.5. In addition, the breakdown of protein in cheese is the result of the action of enzymes from different sources, such as rennet and a variety of microorganisms; each type of organism may contribute a system of proteolytic enzymes. Furthermore, the environment that exists in cheese can not be duplicated in test tube experiments.

Optimum proteolytic activity when *S. lactis* was grown in milk was found at pH 7.0 (9), which was found to be true for the activity of the cell-free extract of *S. lactis*. The low activity at reactions of pH 5.0 and below explains the marked decrease in rate of proteolysis as the pH dropped in a culture not maintained at higher pH levels and helps explain the greatly increased proteolytic activity when the pH is kept from dropping. Failure to find the enzyme in detectable quantities in the cell-free culture medium after organism growth (9) probably is explainable on the basis of the close association of the enzyme with the cell. Whether the enzyme could be freed from the cells by autolysis or bacteriophage lysis, rather than by mechanical disruption, has not been determined. Pasteurization would destroy any enzyme of this type produced during prepasteurization growth.

SUMMARY

Cell-free extracts prepared from *S. lactis* grown in either milk or vitamin-test casein medium, showed the presence of a heat-labile proteolytic enzyme system with optimum activity against milk, casein, and lactalbumin at pH values near neutrality. Some of the characteristics of this enzyme system have been studied. The presence in the broth growth medium of casein and nicotinic acid increased the proteolytic activity of the cell-free extract. The low level of proteo-

lytic activity at pH 5.0 would seem to limit the activity in cheese of most common types and in other acid dairy products.

ACKNOWLEDGMENT

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A FURTHER STUDY OF EFFECTS OF VACUUM LEVEL AND MILKING DURATION ON UDDER HEALTH AND MILK PRODUCTION

R. D. MOCHRIE,[1] H. H. HALE, H. D. EATON, R. E. JOHNSON, AND W. N. PLASTRIDGE
Departments of Animal Industries and Animal Diseases,
Storrs Agricultural Experiment Station, University of Connecticut, Storrs

Poor machine milking practices, especially high vacuum and excessive milking duration, have long been associated with an increased incidence of mastitis (1,6). Dodd *et al.* (3) found a higher incidence of clinical mastitis in first-calf heifers milked for an 8-minute duration than in those milked for 4 minutes.

Previous work from this station (7) reported no significant differences in leucocyte, chloride, and pH values of milk from mastitis-free first-calf heifers milked at vacuum levels of 10, 13, or 17 in. of mercury. The secretion from udder halves milked for a normal duration had significantly greater leucocyte and chloride values and a greater linear rate of increase during the lactation period for leucocyte, chloride, and pH values than did halves milked for a twice normal duration. No significant differences in freedom from mastitis organisms, teat erosions, or udder fibrosis were observed among the vacuum levels or between the milking durations.

This study was undertaken to further evaluate effects of vacuum level and prolonged milking on udder health and milk production. A longer milking period than those used previously was included, and the entire udder was used for each treatment in an attempt to clarify the effects of milking duration.

EXPERIMENTAL

Animals and treatments. In September 1952 six Holstein and six Guernsey cows were selected from 18 animals for having leucocyte counts equal to or less than one million per milliliter in three of four pooled milk samples taken in a 2-week standardizing period, and for being free from mastitis organism in three of these four samples. None of the 12 cows used in the comparison shed mastitic organisms in any of these samples, and the (geometric) mean leucocyte count for all cows was 446,000 per milliliter during this 2-week period. These 12 cows averaged 2.4 $\pm$ 1.4 lactations and had been in milk 175 $\pm$ 63 days at the beginning of the standardizing period. In the standardizing period all cows were milked at 13.5 in. vacuum, and the machines were removed at the operators discretion.

Immediately after the standardizing period, the six cows within each breed

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[1] Present address: Animal Industry Department, North Carolina State College, Raleigh.

were randomly assigned to be milked at one of two vacuum levels for one of three milking durations during a 6-week comparison period. The vacuum levels used were 10 and 17 in., and recorded weekly averages with standard errors were 10.2 ± 0.0, 17.0 ± 0.0 and 10.1 ± 0.1, 16.9 ± 0.6 taken at the line end and teat cup, respectively. The pulsations at these vacuums were 48 ± 1 and 49 ± 1 for the 10 and 17 in., respectively. The milking durations used were "normal," twice normal, and four normal. The "normal" was defined as the length of time from attaching the teat cups to the end of the first 15 seconds during which the rate of milk flow was 0.2 lb. or less. Twice normal and four normal were two and four times the normal for the particular cow. Milking durations were determined, as previously described (7), at both the A.M. and P.M. milkings on the 1st and 22nd days of the comparison period. The averages of these A.M. and P.M. durations were used for all milkings for the 20 days immediately following each determination.

Milking. In both periods, just prior to milking, each cow's udder was washed for 30 seconds with paper towels taken from hot water containing a disinfectant. Then two or three streams were taken from each teat into a strip cup. One minute after the end of stimulation, the machines were attached and the cows were milked as indicated. During the comparison each cow was milked at her assigned vacuum for the predetermined milking duration. All cows were machine stripped after leaving the machines attached for the specified duration.

Feeding. The Holsteins were fed a 16% crude protein grain mixture at the rate of 1 lb. for each 4 lb. of milk, based on the production for the week prior to the standardizing period. This rate of feeding was held constant for the 8 weeks of experimentation. The Guernseys were fed the same grain mixture in a similar manner at the rate of 1 lb. for each 3 lb. of milk. All cows were fed grass hay[2] ad lib and limited amounts of grass silage.

Observations and analyses. Milk weights were recorded twice daily to the nearest 0.1 lb. Twice each week during the standardizing period and once each week during the comparison period, pooled milk samples from each cow (approximately 10 ml. from each quarter) were obtained aseptically. Leucocyte counts to the nearest 10,000 per milliliter, chloride content of these samples, and the presence or absence of mastitis organisms on plates streaked with unincubated milk were determined as described previously (7). Leucocyte counts were converted to three place logarithms of the count times 10^{-4} for the purpose of statistical analysis.

The effects of vacuum level and milking duration on leucocyte count, chloride values, and milk productions were tested in the following analysis of variance:

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[2] A sample taken in the middle of the experimental period was graded U. S. No. 1 timothy hay, exhibited 45% green color, and contained 3.4% foreign material. The plants consisted of 89.3% timothy, 7.3% redtop, 2.3% alfalfa, and 1.1% clover.

Source of variation	*Degrees of freedom*	
Replicates	1	
Vacuum levels	1	
Milking durations	2	
Linear trend among milking durations		1
Residual		1
Vacuum levels × milking durations	2	
Error	5	
Total	11	

In addition to the average response, time trends were explored by separate analyses of first, second, and third order orthogonal polynomial coefficients (4). When accounting for a reduction in variability, the averages for the standardizing period were used to adjust the values obtained in the comparison period by covariance (2).

The values presented in the tables are arithmetic means ± their standard errors.

RESULTS AND DISCUSSION

Leucocytes (Table 1 and Fig. 1). Statistical analysis of the average logarithms of the leucocyte count and the linear rates of change with time (slopes) showed no significant differences between the two vacuum levels or among the milking durations. However, the adjusted averages for the milking durations tended to increase in a linear manner with increasing duration, expressed as logs, ($P < 0.10$). The geometric averages were 461, 761, and 1,130 thousand per milliliter for the normal, twice normal, and four normal durations, respectively.

Chlorides (Table 2 and Fig. 2). As with the leucocyte counts the average chloride values and their slopes over the comparison period did not differ significantly between the vacuum levels. The adjusted average chloride values for milking durations differed significantly ($P < 0.05$) and exhibited a linear increase with increasing time the machines were left on the cows ($P<0.05$). The adjusted average chlorides were 131.7, 147.1 and 163.9 mg.% for the normal, twice normal, and four normal milking durations. The slopes of the chloride values for the milking durations were significantly nonlinear in arrangement ($P<0.05$) with that for the normal duration decreasing slightly, the twice normal decreasing markedly, and the four normal increasing slightly. The durations differed ($P<0.01$) in their tendency to curvature during the comparison period with the normal duration remaining fairly constant except for a slight drop in the final week. The values for the twice normal and four normal durations increased at first, decreased somewhat in the middle of the period, and rose again at the end of the period.

Presence of mastitis. In the 4th week of the comparison period, five hemolytic staphylococcus colonies were cultured from one cow being milked at 10 in. vacuum for the normal duration. The leucocyte count was 520 thousand per milliliter and those for the 5th and 6th weeks were 3,500 and 1,600 thousand per milliliter,

TABLE 1

Effects of vacuum level and milking duration on leucocyte count $\times 10^{-4}$

	Mean log leucocyte count			*Linear orthogonal polynomial coefficients*	
	Standardizing period	Comparison period	Comparison adjusted for standardizing	Comparison period	Comparison adjusted for standardizing
Vacuum levels					
10″	1.5508 ± 0.1768	1.8828 ± 0.1669	1.9901	0.0181 ± 0.0113	0.0257
17″	1.7489 ± 0.0813	1.8473 ± 0.0892	1.7400	0.0059 ± 0.0253	−0.0018
Milking durations					
N	1.8020 ± 0.0408	1.8285 ± 0.1134	1.6637[a]	0.0369 ± 0.0264	0.0251
2N	1.6579 ± 0.1768	1.8903 ± 0.2020	1.8816	0.0101 ± 0.0080	0.0095
4N	1.4896 ± 0.2350	1.8764 ± 0.1884	2.0499	0.0111 ∓ 0.0286	0.0013

[a] Linear trend $P < 0.10$

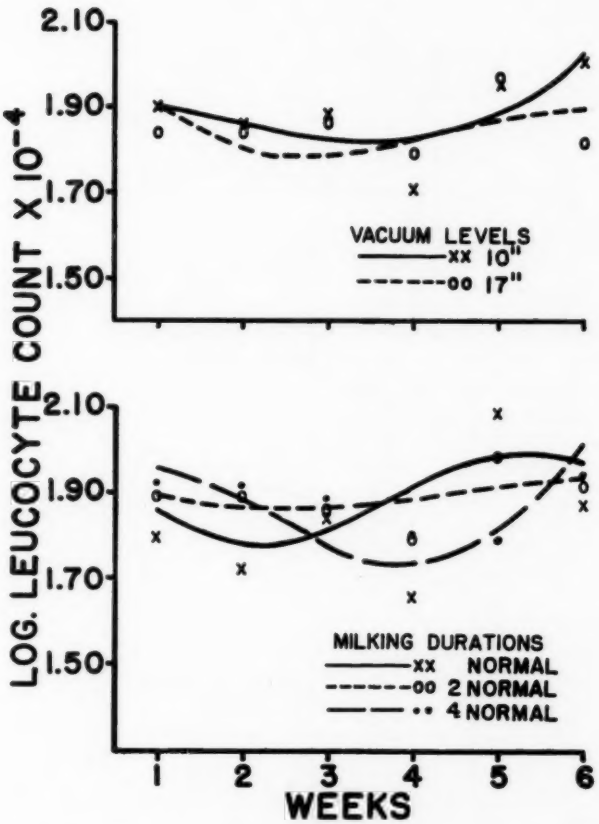

FIG. 1. Effects of vacuum level and milking duration on time trends for leucocyte values.

respectively. No other mastitis organisms were recovered from any of the milk samples drawn in either the standardizing or the comparison period.

No flakes or clots in the milk were revealed by inspection of the strip cup which was used at each milking throughout the experiment. There was no indication of clinical mastitis of any description throughout the trial.

The results reported above indicate that delay in removal of the milking machine after milk flow has ceased can be detrimental to udder health. Although no clinical mastitis developed, the increased chloride and leucocyte values are evidence that some irritation occurred. One possible explanation for the apparent disagreement between these data and the previous report by Mochrie *et al.* (7) may be the relatively short duration of this trial. Given a longer time, these animals might have become adjusted to the disturbance created by the prolonged milking duration. It will be shown later in this report that these cows

TABLE 2

Effects of vacuum level and milking duration on the chloride values

	Mean chloride Mg. %			*Mean linear orthogonal polynomial Coefficient of the chloride value*	
	Standardizing period	Comparison period	Comparison adjusted for standardizing	Comparison period	Comparison adjusted for standardizing
Vacuum levels					
10″	131.4 ± 10.8	147.3 ± 16.0	145.8	−0.9667 ± 1.9610	−1.0553
17″	129.4 ± 7.3	147.8 ± 10.4	149.3	−2.0476 ± 0.6515	−1.9584
Milking durations					
N	127.4 ± 6.2	127.3 ± 8.2	131.7*[a]	−1.0500 ± 0.7568	−0.7793*[b]
2N	139.1 ± 4.6	159.7 ± 12.6	147.1*	−3.6357 ± 1.3036	−4.4096*
4N	124.8 ± 18.0	155.6 ± 21.8	163.9*	−0.1643 ± 2.4867	+0.6684*

* $P < 0.05$

[a] Linear trend $P < 0.05$

[b] Nonlinear trend $P < 0.05$

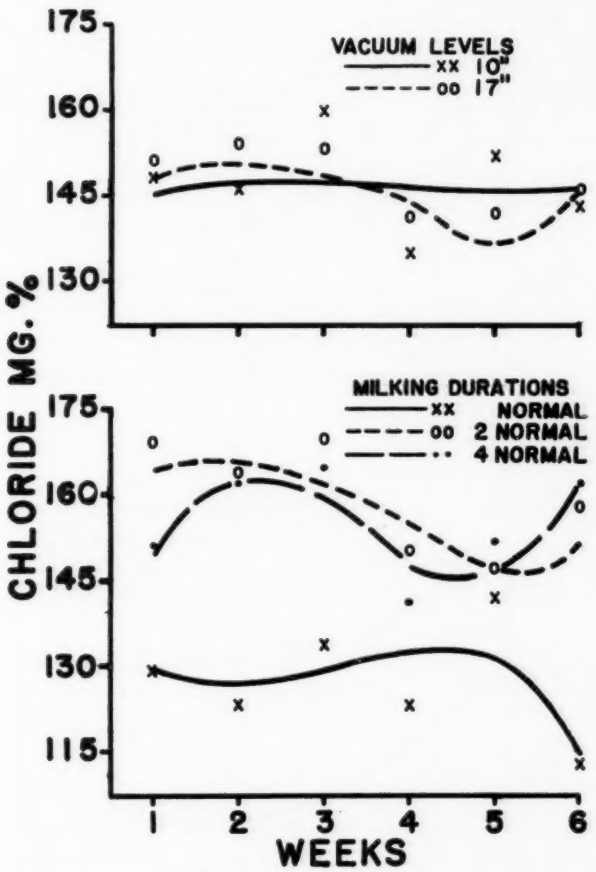

FIG. 2. Effects of vacuum level and milking duration on time trends for chloride values.

did, in fact, become adjusted to the prolonged milking as far as milk production was concerned. It must also be considered that halves of udders may not respond to a particular treatment in the same manner as the whole udder. For instance, Knodt *et al.* (5) reported a highly significant reduction in milk yield from halves of udders milked 12 minutes after stimulation for letdown when compared to control halves milked 2 minutes after stimulation. In contrast, no significant decline in production was noted when entire udders were milked 12 minutes after stimulation as compared with a 2-minute delay.

Milk production. Average pounds of milk produced daily showed no significant differences between the 10 and 17 in. vacuum groups, and the linear rate of decrease in production over the comparison period was approximately the same for the two groups. Although no significant differences in average daily

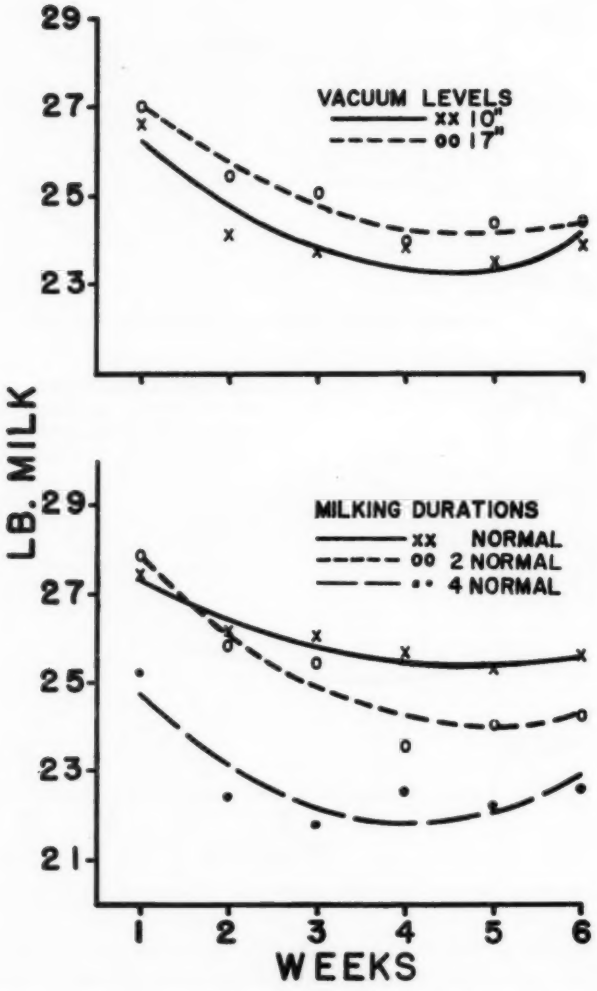

Fig. 3. Effects of vacuum level and milking duration on time trends for daily milk production.

production were noted for the three milking durations, cows in the twice normal and the four normal groups dropped off in production more rapidly in the early part of the comparison. This early drop was offset by greater persistency in the last 3 weeks of the period. The adjusted quadratic coefficients for the milking durations were + 0.1177, + 0.2572, and + 0.3302 for the normal, twice normal, and four normal, respectively, and were significantly arranged in an increasing linear order ($P < 0.05$).

TABLE 3

Effects of vacuum level and milking duration on daily milk production

	Mean daily milk production			*Mean linear orthogonal polynomial coefficient of daily milk production*	
	Standardizing period	Comparison period	Comparison adjusted for standardizing	Comparison period	Comparison adjusted for standardizing
Vacuum levels					
10″	28.2 ± 4.6	24.2 ± 3.8	24.5	−0.4314 ± 0.1675	−0.4336
17″	28.8 ± 5.1	25.0 ± 4.9	24.8	−0.5119 ± 0.2201	−0.5097
Milking durations					
N	29.4 ± 0.7	26.0 ± 0.6	25.2	−0.3328 ± 0.1905[a]	−0.3260[b]
2N	29.1 ± 7.8	25.2 ± 7.8	24.6	−0.7214 ± 0.2156	−0.7169
4N	27.0 ± 7.4	22.8 ± 5.9	24.1	−0.3607 ± 0.2805	−0.3720

[a] Nonlinear trend $P < 0.05$

[b] Nonlinear trend $P < 0.10$

SUMMARY

Six Holstein and six Guernsey cows, free from mastitis in a 2-week standardizing period while being milked at 13.5 in. vacuum, were used in a comparison to determine effects of vacuum level and milking duration on udder health and milk production. They were milked at a vacuum of 10 or 17 in. for a duration of normal, twice normal, of four normal over a 6-week period.

No significant effects of vacuum level on leucocyte count, chloride values, presence of mastitis organisms, or milk production were noted. In contrast, higher leucocyte and chloride values indicated irritation to the udder that was attributed to excessive milking duration.

The cows on twice normal and four normal dropped off more rapidly in milk production when first placed on treatment but were somewhat more persistent later in the trial.

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A METHOD FOR DETERMINATION OF LACTOSE IN MILK BY PAPER CHROMATOGRAPHY

C. J. HONER AND S. L. TUCKEY
Department of Food Technology
University of Illinois, Urbana

Partition chromatography provides a technique for the separation and isolation of compounds of similar structure and properties which at times can be achieved by no other method. Partridge (6) demonstrated that a mixture of reducing sugars could be separated by paper chromatography. Later Flood *et al.* (2) used the same procedure for separation but converted it to a quantitative method by eluting the sugars from the strip and determining them with a microtechnique using a copper reagent. Hawthorne (3) introduced a modification by determining the sugars after elution with periodate oxidation. A direct photometric method was developed by McFarren *et al.* (4). In this method the maximum densities of the developed sugar spots were determined by means of a densitometer. The quantity of sugar was determined from standard curve which related log of sugar concentration and spot densities.

The direct quantitative reduction methods used at present for determining lactose in milk make no allowance for the reducing sugars other than lactose. Moreover, since these methods are not specific for lactose, they are not satisfactory for analyzing certain dairy products such as sterilized milk, cheese, and processed foods containing milk solids in which lactose may not be the only sugar. Whitnah (9) presented evidence that indicated glucose was a normal constituent of raw milk. Later Anantakrishnan and Herrington (1) used a technique which was specific for glucose and confirmed the observation that glucose as well as lactose was present in raw milk.

The purpose of this investigation was to demonstrate that a quantitative chromatographic technique for lactose in milk could be developed which would permit separation of lactose from all other sugars present with its subsequent determination.

METHODS AND EXPERIMENTAL PROCEDURE

The method permits direct application of the milk to the strip; after partition, the sugar spots are located on the chromatogram with the aid of a standard strip containing the pure sugars. In order to eliminate the time required for elution of sugar from the strips, lactose is determined directly. This is done by cutting the chromatogram containing the sugar spot into a series of segments, permitting each segment to react with a solution of alkaline potassium ferricyanide, the composition of which was similar to that described by Park and Johnson (5). The optical density of the resulting ferric ferrocyanide complex is measured with a spectrophotometer. The sum of the optical density values

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of all the segments from a sugar spot, after correcting for the blank, is related to sugar concentration. A quantity value for the sugar is read from a prepared standard curve.

Chromatographic procedures have become well standardized during the past 2 years. A descending partition technique, similar to that of McFarren *et al.* (4) but with modifications, was used for producing the chromatograms.

Equipment:

a. Partitioning chamber — A borosilicate glass cylinder 18 in. in diameter and 24 in. high lined with two sheets of Whatman No. 1 filter paper 18¼ × 22½ in.
b. A solvent trough — A Pyrex baking dish with sloping sides. Top, 9½ × 5¼ in.; bottom, 8 × 3¾ in.
c. A support for the trough — A stoppered 2-l. measuring cylinder.
d. Partitioning columns — Strips of Whatman No. 1 filter paper cut 6 in. wide and 22½ in. long. The long axis was parallel with the water marks.
e. A glass cover for the chamber — A piece of plate glass cut 14 × 14 in. Stopcock grease was applied to the edge of the jar to assure an airtight seal when the glass plate was in place.
f. An insulating container for the chamber — A fiber board box 14 × 14 × 26 in. protected the chromatograms from sudden temperature changes and light.
g. A spectrophotometer — A Beckman Model DU.
h. A paper cutter. This was used to cut chromatograph strips into segments.
i. Pipettes — 0.01 ml. Breed and Brew types.
j. Calibrated test tubes. Tubes were etched at 4 ml. level.
k. Additional laboratory equipment, such as volumetric flasks, analytical balance, test tube rack, separatory funnels, and forceps.

Reagents:

a. Solvent system — 2.5 parts ethyl acetate, redistilled, 1 part pyridine, redistilled, and 3.5 parts distilled water (4). The reagents were equilibrated immediately before use by vigorous shaking in a separatory funnel. The water-rich layer was discarded. Sufficient solvent-rich phase was added to the jar to cover the bottom and the remainder was placed in the trough.
b. Silver nitrate and acetone solution — 5 ml. saturated aqueous silver nitrate (C. P.) plus 95 ml. acetone (C. P.) (8).
c. Potassium ferricyanide solution, reagent — 0.1 g. per 100 ml. distilled water.
d. Sodium carbonate-potassium cyanide solution — 0.8 g. Na_2CO_3 (C. P.) and 0.25 g. KCN (reagent) were dissolved in 100 ml. distilled water.
e. Ferric sulfate and gum arabic — 1.5 g. anhydrous ferric sulfate (reagent), 1 g. gum arabic (USP) and 1 ml. concentrated sulfuric acid (reagent) were diluted to 1 l. with distilled water.

Preparation of standard curve for lactose (anhydrous) C. P. Reagent grade lactose was dried to constant weight by heating it at 80° C. under vacuum.

Five-tenths, 1.0, 2.0, 4.0 and 5.0 g. of lactose, respectively, were dissolved in 1 l. of distilled water. Each 0.01 ml. contained 5, 10, 20, 40 and 50 γ of lactose. Two strips were prepared for each concentration and 0.01-ml. samples were deposited on the strips. The strips were developed and a quantitative determination of lactose was made as subsequently described. One column strip at each concentration was used as a control to locate the lactose spot. The other column of each sample was used for the quantitative measurement of lactose. Ten segments free from sugar were cut from each column strip at time of analysis for a blank. A graph was prepared by plotting optical density readings (ordinate) against concentration of lactose (abscissa).

Chromatographing the sample. A strip of Whatman No. 1 filter paper was cut 6 × 22½ in. for the partitioning column. Two lines, *A* and *B*, were drawn 4 and 5 in., respectively, from one end of the column. Seven points at ¾-in. intervals, were marked on line *B* as sample application spots. The first point was always used for the control or known sample.

Application of sample to chromastrip. Five to 45 γ of lactose per column represents the normal range for analysis by this method. Sixty γ analyzed as subsequently described, developed such an intense color that the optical density value was near the upper working limit of the spectrophotometer. Milk was analyzed for lactose by first diluting a 10-g. sample to 100 ml. with distilled water at 20° C. and then using 0.01-ml. sample to deposit on the strip at a marked point. Approximately 40-45 γ of lactose were present in this volume of sample. It was confined to a small area on the paper by quickly drying it with a stream of warm air blown under the strip. The latter was folded on line *A*, after all samples had been applied, and suspended from the solvent trough along the fold. The short end (4 in.) dipped into the solvent while the remainder hung free in the chamber. The strip was irrigated for 24 hours to secure complete separation and concentration of lactose in a well defined area.

Development of chromatogram and location of lactose. After 24 hours, the strip was removed from the chamber and dried at room temperature for 1 hour. It was cut the entire length midway between the first and second sample application points to form a strip ¾ × 22½ in. This served as the control strip. The latter was dipped into the aqueous silver nitrate-acetone solution for color development and location of the sugar spot. Thus, it served as a guide for locating lactose on the undeveloped portion of the partition column. This latter portion was further dried for 6 hours at room temperature under vacuum.

Quantitative determination of lactose. The dried, undeveloped strip was cut vertically into six columns ¾ × 22½ in. at points equidistant between each sample application. The lactose spot was located on each column by using the developed column as a guide for each chromastrip. The portion of the strip up to ¼ in. of the lactose spot was discarded. The remaining adjacent portion was cut into 10 segments 1/10 × ¾ in. The entire lactose spot plus small blank areas on either side were included in this number. The segments were cut with a paper cutter, the top of which was covered with logarithm paper with the abscissa at 1/10-in. intervals. Each segment was placed with forceps in a num-

bered, clean, dry, calibrated test tube (6 × 150 mm.) and was forced to the bottom of the tube with a clean, dry glass rod. Ten blank segments 1/10 × ¾ in. also were cut from each strip below the lactose spot. Tests showed them to be free from sugar. The blank segments were placed in tubes and treated in exactly the same manner as those from the control and unknown samples. To each sample tube was added 0.5 ml. potassium ferricyanide and 0.5 ml. sodium carbonate-potassium cyanide solutions so that each segment was completely covered. A holder containing all tubes was placed in a bath of boiling water for exactly 5 minutes and then removed and cooled to below 30° C. in another bath maintained at 20-25° C. After 10 minutes the volume of each tube was adjusted to the 4-ml. mark by adding ferric sulfate–gum arabic solution. Tubes were mixed by inverting them. After 15 minutes at room temperature (28-30° C.), the samples were read in a spectrophotometer. Although the color is fairly stable, the samples should be read within 45 minutes after the ferric sulfate–gum arabic solution is added. The instrument was standardized and all readings made at 600 mμ. The blank was made by combining into one all solutions from the 10 tubes containing the blank segments. The readings were recorded as optical density values, since these are additive. The readings for lactose started at a minimum, increased to a peak, and receded again to a minimum. The optical density readings for the tubes containing the lactose spot segments were totaled. This value was related to lactose concentration. The exact quantity of lactose was determined from the prepared standard curve.

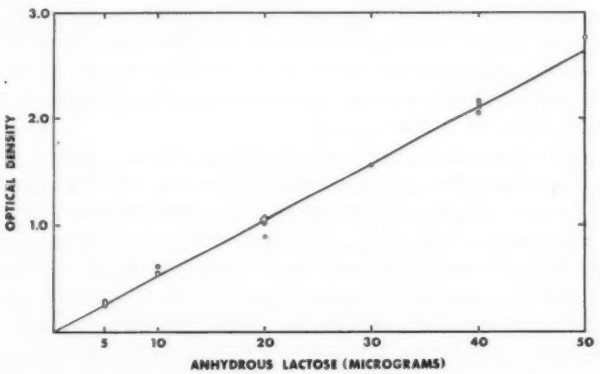

FIG. 1. Relationship between optical density and lactose concentration.

RESULTS

Standard curve. Figure 1 represents the standard curve for lactose and shows the relationship between lactose concentration and the optical density of the ferric ferricyanide solution complex. The data were secured by chromatographing 5, 10, 20, 30, 40, and 50 γ of lactose (anhydrous) and then analyzing the strips for the sugar as described above.

No loss of lactose occurred during chromatographic analysis at low levels. When 5 and 10 γ of lactose (anhydrous) were chromatographed and the results compared with those where an equal quantity of lactose was added directly to tubes containing the ferricyanide reaction mixture, no statistically significant difference was found. Analysis of variance of the results obtained in triplicate, at the two levels of 5 and 10 γ, showed that there was no significant loss of lactose at either level during chromatographic analysis.

Standardization of boiling time. The length of time the tubes containing the sugar and alkaline ferricyanide were kept in a vigorously boiling water bath was determined to be critical. Somogyi (7) stated that at least 8 minutes were required to completely oxidize glucose at boiling temperature in the presence of a copper reagent. His conclusion was based upon a 10-ml. volume of reaction mixture and upon larger quantities of reducing sugar.

Two controlled experiments were undertaken to compare the effect of keeping the reaction mixture in a boiling water bath from 3 to 15 minutes. The increase in optical density values for lactose was linear from 3 to 6 minutes, and beyond 6 minutes the values tended to increase, but in somewhat irregular fashion. A similar trial in which only blank paper segments were used, showed a fairly uniform value from 3 to 6 minutes; with additional time the optical density increased slightly in an unpredictable manner. Furthermore, the paper segments showed some disintegration at 15 minutes of boiling, causing it to interfere with the spectrophotometer reading. Therefore, a standard reaction time of 5 minutes in a boiling water bath was selected. The time was controlled at 5 minutes $\pm$ 1 second by means of a stop watch. A variation of $\pm$ 1 second from the standardized time will contribute 0.5% error in the value obtained for lactose.

Effect of removal of casein from milk. Lactose analysis by chromatography eliminates the necessity of separate protein precipitation if the milk is properly diluted before it is applied to the chromatostrip. Interferences of lactose movement were noted when high concentrations of milk were applied, but this was not true when the concentration of solids was properly adjusted. Excellent separation on the chromatostrip was secured when 0.01-ml. sample of a 1 to 10 dilution of the milk was applied.

When milk was filtered through an ultra filter to remove the casein, no significant difference in the results was obtained when compared with the direct application method. Data are given in Table 1 to illustrate this contention.

Recovery of added lactose. The percentage recovery of pure lactose (anhydrous) from milk is given in Table 2. The results were obtained by the following procedure and calculations:

a. A single sample of pasteurized milk was analyzed for lactose by the chromatographic procedure described under *Methods and Experimental Procedure*. The results are given in Column 1, Table 2. The mean value was 42.87 mg. per gram.
b. One-half the size of milk sample used in *a*, above, plus 0.2500 g. lactose (anhydrous) was diluted to same volume as was used in *a* and analyzed.

TABLE 1

Effect of presence of protein on lactose determination

Treatment	Diluted milk applied	Ultra-filtrate applied
No. of trials	4	4
Av. lactose, anhydrous *(%)*	4.70	4.575

Analysis of variance

Source of variance	Degrees of freedom	Sum of square	Mean square	Variance ratio	Significance
Treatment	1	0.0313	0.0313	5.48	N. S.
Error	6	0.0343	0.00571		
Total	7	0.0656			

TABLE 2

Recovery from milk of added lactose (anhydrous)

Trial	Lactose of milk	½ Mean lactose of milk	Lactose added to milk	Total lactose found	Lactose recovered	Recovery
	(Mg./g.)	*(Mg./g.)*	*(Mg./g.)*	*(Mg./g.)*	*(Mg./g.)*	*(%)*
1	42.46	21.435	25.00	45.87	24.435	97.74
2	43.63	21.435	25.00	44.50	23.065	92.26
3	43.30	21.435	25.00	45.00	23.565	94.26
4	42.50	21.435	25.00	45.50	24.065	96.26
5	42.46					
Mean	42.87					95.13

Therefore, this sample had one-half the original milk lactose, or 0.2143 g. plus C.P. lactose, in approximately an equivalent amount.

c. Percentage recovery was calculated as follows:
Milligrams lactose, anhydrous, found in *b*, minus ½ mg. lactose, anhydrous, in sample *a* is equal to the milligrams lactose, anhydrous, recovered. The quantity of lactose recovered, divided by the amount of lactose added, multiplied by 100 is equal to the percentage of lactose recovered.

Application of the Student's "T" function to the data in Table 2 showed that the recovery of lactose was not significantly low.

Reproducibility of the method. The optimum standard deviation of the method based upon analysis of milk was calculated to be 0.16% based on a mean value for lactose of 4.516%. This value was determined after making eight analyses for lactose on the same sample of milk. The results are given in Table 3. In addition, 10 replicates of a chromatogram were prepared, each of which had applied to it 40 γ of reagent grade lactose (anhydrous). The chromatograms were analyzed for lactose, and the results also are given in Table 3. The optimum standard deviation secured was 0.13%.

DISCUSSION

Direct chromatographic analysis of lactose in milk has the advantage of quantitatively separating this constituent from other reducing sugars and compounds which might be present. The necessity of analyzing specifically for

TABLE 3
Reproducibility of method

Trial No.	Milk, lactose anhydrous	Solution lactose, anhydrous
	(%)	*(%)*
1	4.57	3.85
2	4.46	3.91
3	4.35	4.09
4	4.35	3.89
5	4.74	3.97
6	4.67	3.96
7	4.38	4.01
8	4.61	4.09
9		4.16
10		4.04
Mean ($\bar{X}$)	4.516	4.00
	0.16	0.13

lactose is infrequent, but in a subsequent paper it will be demonstrated that lactose and the monosaccharides undergo marked changes when milk is autoclaved. A chromatographic technique is valuable under these conditions in order to isolate the sugars and to determine quantitatively the extent of the changes.

No comparison of analytical results for lactose in milk by chromatography and the reduction methods was made, since the standard reduction methods are not specific for lactose. Whitnah (9) and Anantakrishnan and Herrington (1) have found glucose to be present in milk; in a subsequent paper it will be demonstrated that there is at least one, and possibly two, other reducing monosaccharides present in milk. Hence, comparing a nonspecific method with one which is specific is not in order. However, the chromatographic procedure as described is time-consuming and would therefore not be an appropriate method for lactose determination in a control laboratory.

SUMMARY

A quantitative method for lactose determination in milk by chromatographic analysis is described. Direct application of milk to the strip and subsequent determination of lactose on the chromatostrip after partitioning are desirable features of the procedure. The reproducibility of the method was determined for lactose in pure solution and in milk. At a mean value of 4.516% lactose, anhydrous, in milk the optimum standard deviation was 0.16%. The recovery from milk of added lactose, anhydrous, varied between 92 and 97% and averaged 95%.

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DIFFERENCES IN SENSITIVITY OF LACTIC STARTERS TO ANTIBIOTICS

C. K. JOHNS

Bacteriology Division, Department of Agriculture, Ottawa, Canada[1]

It is well known that the presence in milk of minute amounts of antibiotics residual from mastitis therapy may seriously interfere with lactic acid fermentations. Of the various antibiotics available, penicillin is still the most widely used [and also the most easily identified through the disc assay method (15)]. Penicillin has been reported (9, 15, 17) to inhibit the lactic starter organisms more than aureomycin, but our tests in 1952 (7) with two starters indicated that the latter compound was more inhibitory. While the Cornell workers (15), working with a single starter, reported a starter activity of 79% in milk containing 0.3 γ aureomycin per milliliter, we obtained only 17%.

It has been suggested (7) that differences in the sensitivity of the respective starters employed might explain these discrepant findings. Katznelson and Hood (9) found partial inhibition of four of 45 unidentified strains isolated from starters in skimmilk containing 0.00625 units penicillin per milliliter, while seven required 0.2 units. Hunter (6) found that in addition to variation within the species, four strains of *Streptococcus lactis* generally required 0.25-0.3 units penicillin per milliliter to bring about the same reduction in acid development as was achieved by 0.1 units with four strains of *S. cremoris*. Others (2, 9, 18, 19) have also reported variations between starters in sensitivity to penicillin. Johnstone (8) likewise noted that starter strains varied in resistance to aureomycin. To determine whether the suggested explanation was correct, the present studies were undertaken.

MATERIALS AND METHODS

A collection of 25 mixed strain dairy starters was obtained from various sources in Ontario and Quebec.[2] The most vigorous 20 of these were then transferred daily in skimmilk sterilized at 15 lb. per 10 minutes. Fresh whole milk, either raw or pasteurized, and known to be free from antibiotics, was obtained as needed from the Central Experimental Farm Dairy. Each lot was subdivided into 250-ml. portions, to each of which 1% of a distilled water solution of antibiotic[3] of the required concentration was added. After overnight refrigeration

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[1] Contribution No. 357.

[2] It was intended to include the starter used by the Cornell workers, but unfortunately it had been discarded (11).

[3] Penicillin G, Lilly; stock solution containing 18.8 mg. in 100 ml. (300 I.U./ml.). Aureomycin HCl, Lederle; stock solution containing 50 mg. (contents of one capsule) in 500 ml. Stored frozen in 4 ml. portions. Dihydrostreptomycin sulfate, Lilly; stock solution containing 28.5 mg. in 10 ml.

at 4° C. these were dispensed in 10-ml. quantities into sterile test tubes and heated to 82° C. for 5 minutes to destroy naturally occurring inhibitory substances (15). After being cooled, tubes of milk were successively inoculated on a 2 minute schedule with 1 ml. of active starter diluted 1:1 with sterile skim-milk. The initial acidity of triplicate tubes was then determined. After incubating in a water bath at 33° C. for 4 hours, contents of each tube, together with 5 ml. of distilled water used to rinse the tube, were titrated with 0.1 *N* NaOH. A control tube of antibiotic-free milk was used for each starter as the basis for calculating the percentage activity (15) in the presence of the antibiotics.

It is generally agreed that *S. cremoris* is more susceptible to penicillin than is *S. lactis*. To determine which species predominated in the mixed strain starters, the four most resistant (to penicillin), and the four least resistant starters were subsequently plated out, and 20 isolates from each tested for ability to grow at 40° C., in 4% salt, at pH 9.2, and to produce NH_3 from arginine (1, 12, 14).

RESULTS AND DISCUSSION

Individual starters varied strikingly in their activity from day to day, despite every effort to maintain uniform conditions. This is illustrated in Figure 1, wherein the variation in acid development of five different starters in milk containing 0.2 units per milliliter of penicillin is shown. Because of this, repli-

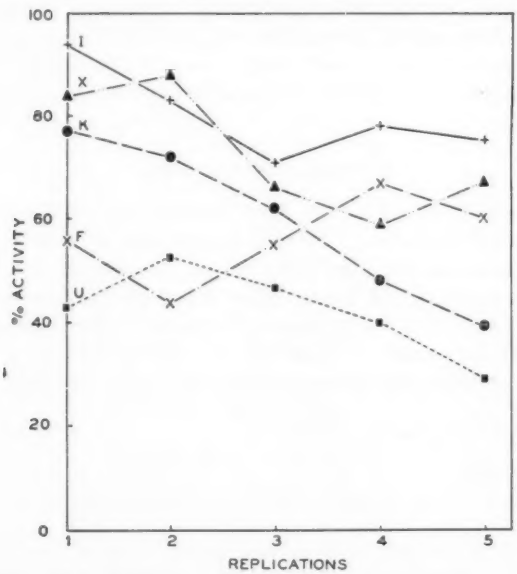

FIG. 1. Illustrating variable results obtained on 5 different days from 5 starters in milk containing 0.2 units penicillin per milliliter.

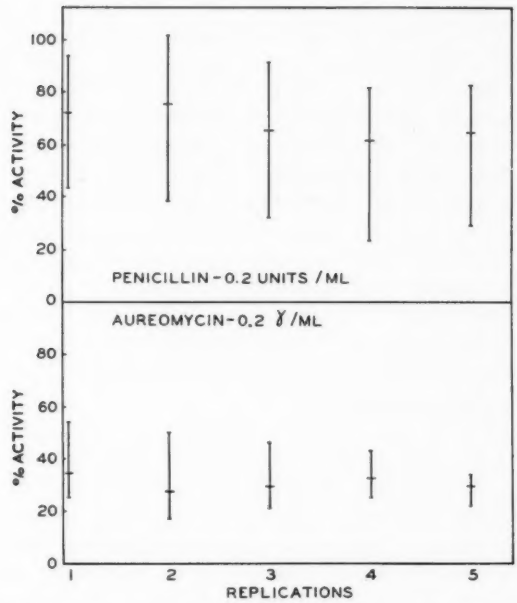

FIG. 2. Maximum, minimum, and mean values for 20 starters in milk containing specified concentrations of antibiotic. Replicate tests on 5 different days.

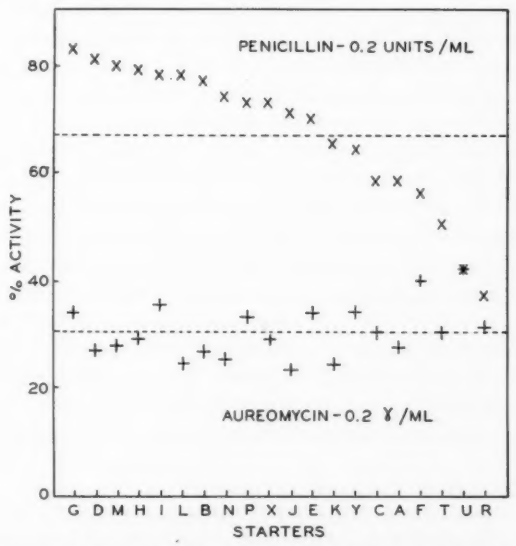

FIG. 3. Comparing sensitivity of starters to penicillin and to aureomycin. Each value represents mean of replicate tests on 5 different days.

cate tests were made on 5 separate days with both penicillin and aureomycin. (A similar number of tests was also run on eight starters using 0.05 γ per milliliter aureomycin). The ranges of maximum, minimum, and mean values for penicillin and aureomycin are shown in Figure 2. It is evident from these data that there are marked differences between starters. Although some overlapping occurs, most starters are appreciably more sensitive to aureomycin than to a similar unit concentration[4] of penicillin.

In Figure 3 the average values from five replications for each of the 20 starters have been arranged in order of increasing sensitivity to penicillin (0.2 units per milliliter), and the corresponding values for milk containing aureomycin (0.2 γ per milliliter) have been plotted. The mean values for all 20 starters are 67% for penicillin and 30.5% for aureomycin. These data show that *(a)* at the concentrations employed here, most starters are decidedly more sensitive to aureomycin than to penicillin and *(b)* there is no relation between sensitivity to penicillin and that to aureomycin.

The sensitivity to aureomycin is considerably greater than that reported by the Cornell workers. Our results suggest that differences in starter sensitivity are unlikely to be the explanation. Grady and Williams (4) recently have warned against using aureomycin hydrochloride as put up for medical use, as it contains excipients. Our first solution, prepared by weighing out 10 mg. from a capsule said to contain 50 mg. aureomycin hydrochloride, gave results indicating approximately 25% of the strength of later solutions prepared by dissolving the entire contents of the capsule. Failure to observe this precaution may explain the lesser inhibitory effects reported by some workers.

When these studies were commenced, it was not our intention to include antibiotics other than penicillin and aureomycin, since these are the two with which slow working of starters has been definitely associated. Some earlier tests with dihydrostreptomycin in concentrations from 0.1 to 0.5 unit per milliliter had yielded results in good agreement with those of the Cornell workers (15), indicating little cause for concern at these concentrations. As the present studies were nearing completion, however, Stoltz and Hankinson (17) reported very strong inhibition with concentrations of streptomycin of 100 units per milliliter and higher. Consequently, a few additional tests were made with dihydrostreptomycin at concentrations (0.4 and 2 units per milliliter) more likely to be encountered in herd milks (16). The results (Figure 4), the average of two replicate tests, show that this compound must usually be present at nearly ten times the concentration of aureomycin to have as great an inhibitory effect, whereas at twice the concentration (0.4 units) it is less inhibitory than penicillin (80.5 vs. 67.0% activity). Comparison with Figure 4 shows little evidence of any correlation between sensitivity to one antibiotic and that to either of the

[4] It is realized that since 1 unit penicillin represents only 0.6 γ while 1 unit of aureomycin represents 1 γ, the concentration by weight of aureomycin at a given unit concentration is 66.7% greater. On the other hand, since the actual number of molecules present per unit concentration is of primary importance, the lower molecular weight of penicillin (356.38 compared with 508 for aureomycin) largely compensates for its lighter unit (100:117).

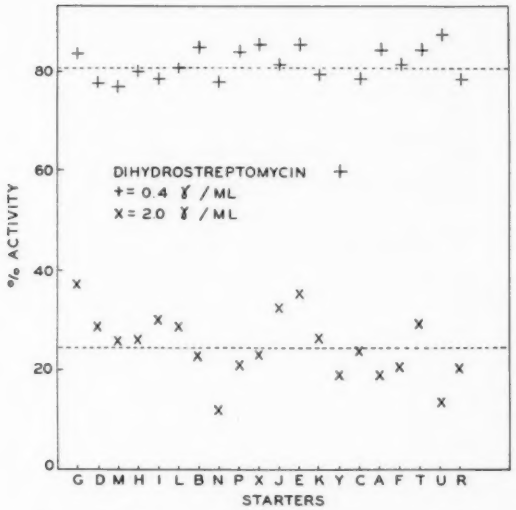

FIG. 4. Sensitivity of starters to 2 concentrations of dihydrostreptomycin. Each value represents mean of 2 replicate tests on different days. (Starters arranged in same order as in FIG. 3.)

others. Furthermore, results with the two different concentrations sometimes failed to agree, e.g., Starter U. This was also observed when comparing aureomycin at 0.05 and 0.2 units per milliliter (Figure 5). It seems peculiar that starters should behave so differently in the presence of different concentrations of the same antibiotic.

Difficulty was experienced in identifying strains isolated from the eight starters as either *S. lactis* or *S. cremoris*. In very few instances did cultures show characteristic behavior (14). Even when grouped on the basis of two or more characteristics, wide variations in the percentages classed as *S. lactis* were noted for the more resistant starters; the same held true for the percentages of *S. cremoris* in the less resistant ones (Table 1). In view of the clear-cut differentiation reported by other workers, this is difficult to understand. These data show no definite indication of a correlation between predominant species and resistance to penicillin.

The data recorded in Figure 3 confirm the reported variations in sensitivity of starters to penicillin (2, 6, 9, 18, 19). They do not, however, explain the disparity between the level of sensitivity reported by Hood and Katznelson (5), i.e., 1:330,000,000 or 0.003 units per milliliter, and that reported by Turton and Pearce (19), i.e., "the presence of 0.5 I.U. per milliliter resulted in the production of slightly less acid than . . . 0.05 I.U. per milliliter, but sufficient acid was produced to allow normal setting." Similarly, our data for aureomycin (Figure 3) fail to explain the wide range of sensitivity reported. Overby (13) stated that 0.02 γ per 100 ml. (sic) retarded the development of a mixed strain

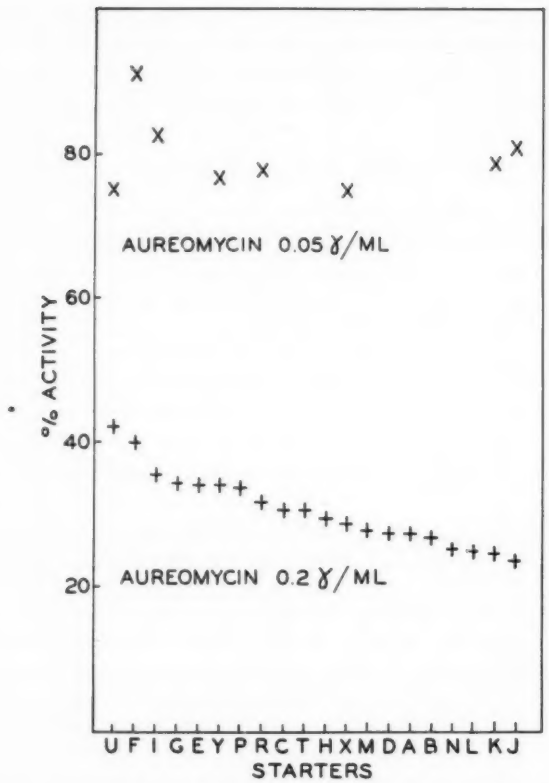

FIG. 5. Relative inhibition induced by 2 concentrations of aureomycin in milk. (Each value is the mean of determinations on 5 different days. Starters arranged in order of increasing sensitivity to 0.2 γ aureomycin per milliliter.)

TABLE 1

Tentative classification of isolates from lactic starters (20 isolates from each starter)

Starter	Classification[a]	S. lactis[b]	S. cremoris[c]	Intermediate
		(%)	*(%)*	*(%)*
D	R	55	15	30
F	S	35	20	45
G	R	60	15	25
H	R	20	45	35
M	R	20	70	10
R	S	30	30	40
T	S	30	45	25
U	S	40	50	10

[a] R = Resistant to penicillin.
S = Susceptible to penicillin.

[b] If positive on 2 or more of 4 diagnostic tests (growth at 40° C., at pH 9.2, in 4% NaCl and production of NH_3 from arginine), culture classed as *S. lactis*.

[c] If negative on 3 or more, classed as *S. cremoris*.

starter, but Bradfield *et al.* (3) contended that "there was very little evidence of inhibition of starter activity when the amount of aureomycin was less than 0.25 γ per milliliter." Some of these discrepancies may stem from difficulties in preparing standard solution or in calculating concentrations.

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SEPARATION OF SODIUM, POTASSIUM, MAGNESIUM, AND CALCIUM IN MILK ASH BY ION-EXCHANGE CHROMATOGRAPHY

WILLIAM J. L. SUTTON AND EMORY F. ALMY

The Department of Agricultural Biochemistry and The Institute of Nutrition and Food Technology, The Ohio State University, Columbus

In the past few years there has been increased use of the flame photometer in the determination of alkali metals (2, 3, 5, 8, 9). Brown *et al.* (2, 3) described its use for the determination of sodium and potassium in plant extracts and for calcium and magnesium in leaves. They observed the mutual interference of sodium and potassium and the influence of sodium on the photometer reading for calcium. Fox (5) used a lithium internal standard in the determination of sodium and potassium in blood and urine. A recent paper on flame photometry (9) suggests the use of standards of the approximate composition as the unknown to avoid the effects of extraneous elements on the intensity of emission of the test element.

Keirs and Speck (8) used a photometer for determination of sodium, potassium, and calcium of milk ash. They found there was mutual interference of sodium and potassium and minimized the effect by the use of standards and unknowns with approximately the same concentration of interfering element. They were unable to determine magnesium by flame photometry.

This paper offers a procedure using ion-exchange chromatography for the complete separation of these chemically similar cations. It avoids the errors due to mutual interference of ions in flame photometry procedures; uses simpler, less expensive equipment, and is sufficiently accurate to determine these elements in milk to $\pm$ 2%.

Ion-exchange chromatography has become increasingly important as a technique for separation of chemically similar cations. Previous investigations (4, 6, 7) have indicated that under certain conditions ion-exchange separation of sodium and potassium is possible. In recent papers (1, 10) Dowex-50 (a nuclear sulfonic acid cation exchanger) was reported to have been used to separate and determine these two elements.

The work reported in this paper, using colloidal Dowex-50, extends the method of Beukenkamp and Rieman (1), for the separation of sodium and potassium, to obtain a complete simultaneous separation of sodium, potassium, magnesium, and calcium in salt mixtures and in milk ash. As far as the authors are aware, there is, at present, no ion-exchange procedure for the separation of these four elements in biological materials.

The procedure herewith reported uses 0.7 *N* hydrochloric acid for elution and separation of sodium and potassium from a cation exchanger column, which retains magnesium and calcium ions. The divalent ions are subsequently eluted from the column and separated using 1.0 *N* hydrochloric acid.

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APPARATUS AND REAGENTS

The ion-exchange column used is essentially that described by Beukenkamp and Rieman (1). Their procedure for resin treatment was followed. The resin bed was supported on a fine grade filter disk fitted by careful grinding to size into the lower end of a Pyrex glass tube approximately 23 mm. in internal diameter. Preliminary experiments indicated that a 61-cm. column of resin (colloidal Dowex-50, batch L 2111-41) was required for the separation.

All chemicals used in the preparation of salt mixtures for column separation were of reagent grade; viz., sodium chloride, sodium citrate, potassium chloride, di-potassium hydrogen phosphate, magnesium carbonate, calcium carbonate, hydrochloric acid, and lactose.

EXPERIMENTAL PROCEDURE

Preparation of samples. Synthetic mixtures containing sodium, potassium, magnesium, and calcium were prepared by dissolving weighed amounts of their salts in distilled water. Sodium was used as chloride and citrate, potassium as potassium chloride and di-potassium hydrogen phosphate. Magnesium and calcium were weighed as carbonates and converted to chlorides by a slight excess of 0.7 *N* hydrochloric acid.

In the preparation of milk ash samples, 100 g. of a well-stirred sample of whole milk were weighed into a large porcelain casserole. A few drops of glacial acetic acid were added. The milk was well stirred and evaporated to dryness on a steam bath. The residue was ignited in a muffle furnace at 550° C. until a white ash remained. The ash was extracted in 100 ml. of 0.7 *N* hydrochloric acid.

Column operation. The resin was first completely converted into the hydrogen form by running 0.7 *N* hydrochloric acid through for 3 to 4 hours at a flow rate of about 2.5 ml. per minute. The acid was allowed to drain down until level with the top of the resin bed. The sample was added by pipette to the top of the resin bed (generally the sample volume was 10 ml.). This solution was again slowly drained down until level with the top of the resin bed.

Separation of sodium and potassium. Hydrochloric acid (0.7 *N*) was allowed to run slowly over from the acid reservoir to give a layer a few centimeters deep above the resin. Input from the reservoir and outflow from the column then were adjusted to maintain this volume above the bed with an effluent flow rate of 2.3 ml. per minute. The first 300 ml. of eluate were collected in a graduated cylinder. This was always found free from any metallic cations.

The eluate then was collected in fractions (8 ml. or 10 ml.) up to 700 ml., and each fraction was tested for sodium and potassium. A simple flame test on each fraction will enable the operator to determine approximately the limits of sodium and potassium elution. The elution of potassium was found to be complete at 700 ml.

Elution of magnesium and calcium. Elution by 0.7 *N* hydrochloric acid was discontinued after 750 ml. of eluate had been collected. Elution of magnesium and calcium with 1.0 *N* hydrochloric acid then was commenced, and eluate frac-

tions were collected from 800 ml. to 1,600 ml. in volume. Elution of both magnesium and calcium was found to be completed under these conditions at about 1,500 ml.

Separation and analysis of mixtures. The procedure for the separation and determination of sodium and potassium in salt mixtures and in milk ash employed the same method of elution of sodium and potassium by 0.7 *N* hydrochloric acid. Eluate from 0 ml. to 300 ml. was discarded; that from 300 ml. to 500 ml. contained all the sodium; and that from 500 ml. to 700 ml., all the potassium. Each of these 200-ml. fractions was collected in graduated cylinders. After mixing thoroughly, 25-ml. aliquots of each were analyzed.

In the above procedure the eluate range free of metal cations will depend on the particular batch of colloidal Dowex-50 used in the column (5). The eluate should, therefore, be collected in small fractions in preliminary runs to establish separation limits in eluate volumes for each cation before carrying out quantitative determinations.

ANALYSIS OF ELUATE FRACTIONS

The eluate fractions were evaporated to dryness on a steam bath to remove all uncombined hydrochloric acid, and each residue was extracted in 50 ml. of distilled water.

A comparison of "titration blanks" of *(a)* distilled water and *(b)* 0.7 *N* hydrochloric acid from the column (in the range 0 ml. to 300 ml.), evaporated to dryness and extracted as before, indicated complete removal of free hydrogen chloride. Chloride ion in each treated, extracted fraction was determined by the Mohr chloride titration procedure with 0.02 *N* silver nitrate. Fifty ml. of distilled water was used for determining an indicator "blank." Any fraction giving a Mohr titration not greater than that with the distilled water indicator blank was considered free of metal chloride. The amount of metal cation in each fraction was determined.

RESULTS AND DISCUSSION

The data tabulated in Table 1 indicate that sodium and potassium can be separated on a 61-cm. column of Dowex-50 under the conditions used. Improved separation is obtained by decreasing sample volume. A 10-ml. sample was used in all further elutions (unless stated otherwise).

It was considered advisable to determine whether certain constituents normally occurring in whole milk, e.g., phosphate and citrate ions and lactose, had any effect on the ion-exchange separation of sodium and potassium.

In Table 2, sodium and potassium concentrations in salt mixtures used approximate those normally found in milk. In elutions No. 4, 5, and 6 (Table 2), each 10-ml. aqueous sample of alkali cations contained, in addition to the amounts of anions tabulated, 20 mg. of citric acid and 500 mg. of lactose. These added constituents have apparently little effect on the separation.

TABLE 1

Effect of sample volume on the separation of sodium and potassium (Sodium eluted first)

Eluate volume (ml.)		Sample volume, milliliters 25	10	5
		(Amount of alkali cation)		
		(mg.)	(mg.)	(mg.)
464-468		0.4	0.4	0.3
468-472	Na decreasing	0.2	0.2	0.2
472-476	K absent	0.1	0.0	0.1
476-480		0.1	0.0	0.0
480-492		0.0	0.0	0.0
492-496		0.1	0.0	0.0
496-500	K increasing	0.3	0.1	0.0
500-504	Na absent	0.7	0.5	0.1
504-508		1.3	0.7	0.3

Sample: 60.0 mg. sodium, and 140.0 mg. potassium (as chlorides).
Flow rate — 0.60 ml./min./sq.cm.
Elutrient solution — 0.7 *N* HCl.
Temperature — 25° C.

TABLE 2

Separation of sodium and potassium

Number	Quantity taken Na	K	Corresponding anion of alkali metal Na	K	Range of eluate volume in which cation detected Na	K
	(mg.)	(mg.)			(ml.)	(ml.)
1	15.0	35.0	Cl^-	Cl^-	368 to 480	594 to 656
2	6.0	14.0	Cl^-	Cl^-	368 to 464	528 .....
3	6.0	14.0	Cl^-	Cl^-	376 to 472	536 .....
4	6.0	14.0	Citrate	HPO_4^{--}	376 to 480	536 to 672
5	6.0	8.5	Cl^-	HPO_4^{--}	376 to 488	544 to 656
6	6.0	8.5	Cl^-	HPO_4^{--}	384 to 472	544 to 648

Elutrient solution — 0.7 *N* HCl. Eluate collected in 8-ml. fractions.
Flow rate — 0.60 ml./min./sq.cm.
Temperature — 25° C.
Sample volume — 10 ml., (25 ml. in run No. 1 only).

Elution data summarized in Table 3 show that magnesium and calcium ions, at concentration levels approximating those of milk, do not prevent the separation of sodium and potassium in salt mixtures of the four elements, or in milk ash.

It was considered that since monovalent sodium and potassium were completely eluted from the resin column by 0.7 *N* hydrochloric acid, continued elution with hydrochloric acid of greater molarity should remove divalent magnesium and calcium.

A number of experimental runs and analyses of 10-ml. eluate fractions from 800 ml. to 1,600 ml. finally gave an elution and separation of magnesium and calcium with 1.0 *N* hydrochloric acid as the elutrient.

Typical data for an elution and separation of the four cations in the ash from 10 g. milk are summarized in Table 4, and the elution curves are shown in Figure 1. The elution by 1.0 *N* hydrochloric acid was started after 750 ml. of

TABLE 3

Effect of magnesium and calcium on the separation of sodium and potassium

Number	Amount taken	Temperature	Range of eluate volume free of all metallic cations
	(mg.)	*(° C)*	*(ml.)*
1	Na 6.0 K 14.0	24	472 to 536
2	Na 6.0 K 14.0 Mg 4.0 Ca 15.0	35	472 to 536
3	Na 6.0 K 14.0 Mg 4.0 Ca 15.0	31	480 to 536
4	Milk ash from 10 g. whole milk	32	488 to 520

Elutrient solution — 0.7 *N* HCl. Eluate collected in 8-ml. fractions.
Flow rate — 0.60 ml./min./sq.cm.

eluate had been collected. Under these conditions magnesium first appears in the eluate at about 880 ml., but at least 90% of the magnesium is eluted between 1,000 and 1,120 ml. Elution curves showed complete separation of each individual cation.

Data given in Table 5 indicate that the separation procedure permits the accurate determination of sodium and potassium in milk (and probably the accurate determination of calcium), since the chromatographic procedure gives a complete simultaneous separation of each element.

Elutions in Table 5 were carried out at room temperature (26 to 30° C.) and at flow rates in a range of 0.52 ml./min./sq.cm. to 0.56 ml./min./sq.cm. each tabulated value of sodium and potassium found is the mean of three separate analyses, which showed close agreement. The range of each group of three analyses never exceeded 0.2 mg. of cation. Using a 10-g. (or 10-ml.) sample of milk, the error in such sodium or potassium determinations is of the order of 1 or 2 parts per hundred.

TABLE 4

Separation of sodium, potassium, magnesium and calcium of milk ash

Cation	Range of eluate volume in which cation was detected	Amount of cation found
	(ml.)	*(mg.)*
Sodium	370 to 490	4.7
Potassium	520 to 650	8.4
Magnesium	880 to 1120	1.1
Calcium	1220 to 1500	13.3

Elutrient solution — 0.7 *N* HCl for Na-K separation, 1.0 *N* HCl for Mg-Ca separation.
Flow rate — 0.60 ml./min./sq.cm.
Temperature — 30° C.
Eluate collected in 10 ml. fractions.

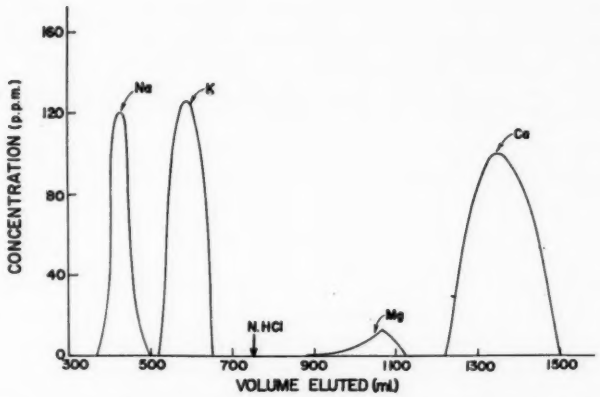

FIG. 1. Separation of sodium, potassium, magnesium, and calcium in milk ash.

TABLE 5

Separation and recovery of sodium and potassium (all weights expressed in milligrams)

Number	Sample taken (10 ml. volume)	Found
1	Na 6.0 Mg 4.0 K 14.0 Ca 15.0	Na 6.1 K 14.2
2	Na 3.0 Mg 2.0 K 7.0 Ca 7.5	Na 3.2 K 7.3
3	10 ml. milk ash extract	Na 5.5 K 11.1
4	10 ml. milk ash extract	Na 5.7 K 11.1
5	5 ml. milk ash extract + 5 ml. H_2O	Na 2.9 K 5.8
6	Na 3.0 Mg 2.0 K 7.0 Ca 7.5 in 5 ml. + 5 ml. milk ash extract	Na 5.8 K 12.8

The foregoing investigation suggests the following general procedure for the separation and determination of these four elements in milk (ash or whey) and other dairy products.

(a) Select a sample weight of material and a volume of 0.7 *N* hydrochloric acid for extraction of its ash, so that 10 ml. of the acid extract contains approximately the same amount of each of the four cations as used in the above method.

(b) Chromatograph 10 ml. of this extract on a column (as previously described) and determine the limiting ranges of respective elution bands (collecting 10 ml. fractions of eluate).

(c) Using the data of *b*, chromatograph a 10-ml. extract, elute, and collect the four cation fractions as each is successively eluted.

(d) Evaporate eluate fractions to dryness on a steam or water bath to remove all uncombined hydrogen chloride. In *b*, use 10 ml. eluate fractions, in *c* use a suitable (25 or 50 ml.) aliquot of each of the four cation fractions.

(e) Extract each residue in 50 ml. distilled water and titrate the chloride with 0.02 molar silver nitrate (Mohr procedure).

(f) Correct each titration for the indicator blank (50 ml. distilled water) and calculate the cation present in each fraction.

SUMMARY

A simple method is described for the separation of sodium, potassium, magnesium, and calcium in salt mixtures, milk ash, etc., by ion-exchange chromatography. After the solution containing the sample on the ion-exchange column is chromatographed, the elution volume ranges for each constituent element are determined in a preliminary trial. The constituent elements then are eluted as chlorides by suitable concentrations of HCl, collecting the eluate volume fractions for the respective cations in the volume ranges found in the preliminary study. Aliquots of each fraction are evaporated to dryness to expel uncombined HCl and then taken up with distilled water. The amount of cation-bound chloride then is determined by the Mohr titration with 0.02 *N* $AgNO_3$.

ACKNOWLEDGMENT

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

EDWARD K. SLATER, chairman of the editorial board of the Olsen Publishing Company and editor of Olsen Publications for 32 years, was born in a log cabin on a farm near Northwood, Iowa, on June 28, 1877. He attended country school and went to high school for two years at Fairmount, Minn. During those early years he worked as a farm hand, taught country school, and got his start in the publishing business as a printer's "devil" and later as a full time reporter on the Fairmount Daily Sentinel.

E. K. Slater

In April 1898, as a member of the Second Regiment of the Minnesota National Guard, he was sworn into service as a second lieutenant in the 12th regiment of the Minn. Volunteer Infantry.

After he was mustered out of service at the close of the war, he took the job of helper in his brother's creamery. Later he attended the short course for experienced buttermakers, after which he became buttermaker in a southern Minnesota cooperative creamery. He operated three different creameries, and on February 1, 1903, was appointed a state creamery inspector by the state Dairy and Food Commissioner. While inspector, he was made secretary of the Minn. Creamery Operators and Managers Association and began his publishing work as editor of the Dairy Record, the Association paper. He organized the Dairy Record Publishing Company, and two years later, when he was appointed commissioner of the Minn. State Dairy and Food Department, he turned over his stock in the Dairy Record to the Buttermakers Association.

Only 28 years old when he took over his duties as the commissioner, he was the youngest agricultural commissioner in the country. During his service from January 1, 1905, to December 31, 1908, many of the state pure food and drug laws were enacted. After serving his term as commissioner, he became Assistant Professor of Dairying at the Minn. College of Agriculture, serving in 1909 and 1910. He obtained a leave of absence in 1909 to be secretary of the National Dairy Union and lobbyist for the dairy industry at Washington during the 1909-1910 session of the United States Congress. He also served one term as manager of the National Dairy Show, a forerunner of the present Dairy Industries Exposition.

For the next ten years Mr. Slater did public relations work for the Blue Valley Creamery Company, with headquarters in Chicago. During this time, he helped organize the National Dairy Council in Chicago.

Earlier, when E. K. was an instructor in the dairy school, he met a young man who had recently arrived from Denmark. The newcomer was H. P. OLSEN. A deep friendship developed between the two men, and when Mr. Slater became commissioner of agriculture, he appointed Mr. Olsen to take over the operation of the Dairy Record Publishing Company. In 1910 Olsen went to Milwaukee and established the Olsen Publishing Company. In 1920 he prevailed upon Slater to join the staff as the editor-in-chief, which position he held until January 1, 1952, when he was made chairman of the editorial board.

E. K. Slater has been recognized for his sound judgment and his ability to properly evaluate problems facing the industry. He helped work out plans for establishment of the Milwaukee Dairy Council and also was active in the organization of the Wisconsin Dairy Technology Society, of which he was the first president.

At present Mr. Slater spends much of his time at his home — Calhoun Farms, Waukesha County, Wis.

Completed Theses

M. S. Degree:

LEONARD F. BLANTON—The application of microscopic examinations in determining the effects of stabilizers and emulsifiers upon the texture of ice cream. N. C. State College.

LEWIS T. SMITH—Genetic variations and covariation in size and production in dairy cattle. N. C. State College.

HAROLD A. RAMSEY—Effect of diet on the diurnal variation of blood plasma lipides in dairy calves. N. C. State College.

MOHAMED SALAH EL-DIN BARRADA—Heat tolerance studies with lactating dairy cattle involving coordinated field and chamber trials. N. C. State College.

HUGH A. POSTON—The effect on lactating dairy

cows of replacing hay with corn silage. N. C. State College.

PHILLIP G. KEENEY—Certain factors affecting the concentration of heat-produced sulphydryl compounds in cream. Ohio State Univ.

PAUL M. LARGE—Characteristics of a sweetened concentrated milk product as affected by processing and storage conditions. Ohio State Univ.

ELEANOR T. BILLIG—Baking qualities of butter as affected by the manufacturing process and the addition of an emulsifying agent. Ohio State Univ.

SARAH I. JENCKS—Consumer reaction to milk varied in fat content and solids-not-fat composition. Ohio State Univ.

W. M. ADAMS—The apparent digestibility of rations containing non-protein nitrogen compounds when fed to dairy steers. Univ. of Kentucky.

T. B. HAMBLIN—The effect of substituting urea or decyandiamide for part of the protein in the grain ration of lactating cows. Univ. of Kentucky.

GARLAND M. BASTIN—The effect of adding ground hay to dairy cattle rations. Univ. of Kentucky.

JAMES R. PERKINS—A study of the genitalia from 1,000 slaughter house cows. Univ. of Kentucky.

Ph.D. Degree:

S. H. DALAL—Some properties of the fat globule interface as related to lipolysis in milk and cream. Oregon State College.

C. J. HONER—The isolation of intermediates in the Krebs citric acid cycle in cheese and fermented milks. Univ. of Illinois.

News from the Departments

North Carolina:

W. E. SANDINE, a winner of the Dairy Industry Supply Association scholarship, is taking graduate work in dairy manufacturing after spending two years in the Air Force.

Designed to encourage superior high school graduates to select a major in dairy manufacturing and to furnish an incentive for the second and third year students who enroll in this curriculum, the North Carolina Dairy Products Assoc. has adopted a student scholarship plan. Two $500 freshman scholarships will be open to residents of the state each year for a period of 3 years, and two $300 and two $200 scholarships will be open to sophomore and junior students each year. The awards will be made on the basis of scholarship, leadership, and need.

R. B. CASADY is taking a year's leave of absence to accept a visiting professorship at Johns Hopkins Univ. to do research work in the field of climatology. Dr. Casady will be replaced on a temporary basis by W. E. THOMAS, who received his Ph.D. degree at Cornell. His work will be in the field of physiology. Dr. Thomas recently was manager of Big Stone Farms, Milford, Del.

Montana:

The 18th Dairy Industry short course was held Nov. 16-18, a dairy Herdsman short course Nov. 9-18, and an Inseminators short course Nov. 19-21.

New Jersey:

JOSEPH G. LEEDER, Professor and Research Specialist in Dairy Manufactures at Rutgers Univ., has recently returned to the United States from Tel Aviv, Israel, where he spent several weeks at the new ice cream plant of Matokar, Ltd. as a consultant on ice cream making. The Matokar company began operations in the middle of August and at the present time is specializing in frozen stick confections. Dr. Leeder's most important job was to draw up formulas for ice cream which would economically utilize the particular ingredients available in Israel. In addition to this, Dr. Leeder advised the plant employees and the management on the proper operation of the plant.

While in Israel Dr. Leeder spent a few days seeing the interesting sights around the country. These trips included visits to Jerusalem, Nazareth, Liberias (the Lake of Galilee), and Haifa.

Maine:

NORMAN NESS has been appointed Extension Service Dairy Specialist to replace RICHARD TALBOT, who retired in July. Mr. Ness, a graduate of the Univ. of Maine, was formerly county agent of Franklin County.

MERTON POORE has been appointed Graduate Assistant in Animal Industry. He is a graduate of the Univ. of Connecticut. His work will have to do with a study of long-time storage of semen at low temperatures.

Vermont:

HENRY ATHERTON joined the Animal and Dairy Husbandry Department staff July 1 as Assistant Professor in Dairy Manufactures. He is a Univ. of Vermont graduate and recently completed requirements for his doctorate at Pennsylvania State College.

ROBERT FITZSIMMONS is on leave studying for his doctorate in animal breeding at Rutgers Univ.

The last Vermont legislature created a State Dairy Commission to promote the sale of milk and dairy products within the state. HUGH RIDDELL, head of the Department of Animal and Dairy Husbandry, is one of the members appointed by the Governor.

Ohio:

The fifth annual Dairy Technology homecoming "brunch and social hour" was held October 31. About 150 alumni and guests attended.

Nine undergraduate students are enrolled in Dairy Technology this fall as a result of a scholarship program sponsored by the industry groups of the State.

The Department of Dairy Technology supervises the displays and activities of the Dairy Products Division at the Ohio State Fair. The objective of this project is to acquaint the public with the dairy industry and its products. This year the highest quality prizes were offered in butter workmanship, and in Swiss, cheddar, and cottage cheese contests. Educational exhibits included the processing of powdered milk, Swiss cheese manufacture, and other exhibits in line with the theme of Ohio's sesquicentennial year which showed developments in the dairy industry in the last 150 years.

R. B. DOUGLAS, Dairy Technology Extension, has taken a position with Beatrice Foods Co., New Bremen, Ohio.

K. K. Fox (M.S. Ohio State, 1952) has been appointed Research Associate in the Ohio State Univ. Research Foundation and is working on proteins of nonfat dehydrated milk, a project sponsored by the Quartermaster Food and Container Institute.

K. SHAHANI, Bombay, India, has been appointed Research Associate and is working on antibiotics in milk, a project sponsored by the U. S. Public Health Service.

Industry meetings:

November 9-20—Milk Short Course
January 11-22—Ice Cream Short Course
February 9-12—Annual Dairy Technology Conference
March 15-19—Milk Sanitarians Short Course

Kentucky:

The first annual Dairy Manufacturing Short Course was given at the Univ. of Kentucky on November 17 and 18, 1953, under the joint sponsorship of the Dairy Section and the Dairy Products Assoc. of Kentucky. The staff of the Dairy Section was augmented by several outstanding dairy technologists from other schools and from the dairy industry.

Restoration of the dairy barn, which was severely damaged by fire earlier in the year, has been completed.

LINVILLE BUSH, Field Agent in Dairying, began his duties in the Extension Division on Sept. 8. He is in charge of the state D. H. I. A. program, replacing JOHN COUEY, resigned. Linville, a graduate of the Univ. of Kentucky, obtained his M.S. degree from Ohio State Univ. He was employed during 1950 in field work in artificial breeding, after which he served in the U. S. Air Force until a short time before assuming his duties at U. K.

JAMES R. PERKINS, Assistant Professor of Dairy Husbandry and Assistant Dairy Husbandman, is on a temporary appointment to fill the vacancy created by the absence of DURWARD OLDS, who is on sabbatical leave. Perkins was granted the M.S. degree by the Univ. of Kentucky in June 1953. He will conduct research and teach in the field of artificial breeding.

W. M. ADAMS, M.S. 1953, has accepted a teaching position at Western Kentucky State College, Bowling Green.

F. B. HAMBLIN, M.S. 1953, has accepted a teaching position at Tennessee Polytechnic Institute, Cookeville.

Cornell:

EDMUND MASUROVSKY, a senior in the Cornell Univ. Dairy Industry Department, recently won the Milk Industry Foundation Collegiate Leadership Award for the northeastern region. This award is made annually to a senior in dairy manufacturing. The scores are based upon the extra curricular activities and scholastic record of the student during his first 3 years of collegiate work.

Oregon:

ROGER W. MORSE has retired as Extension Dairy Specialist at Oregon State College, a position he has held since 1930. Morse was graduated from the State College of Washington. He served as a county agent in Wyoming and Oregon before assuming the position of Dairy Specialist. He and Mrs. Morse will continue to make their home in Corvallis.

The vacancy created by Morse's retirement will be filled by HAROLD P. EWALT, member of the extension dairy staff since 1944. DON ANDERSON will succeed Ewalt.

G. H. WILSTER has returned to his duties after an illness of nearly nine months. He is not fully recovered but is in his office daily.

The Oregon Dairy Breeders Assoc. held its annual meeting October 9 on the campus at Oregon State College. H. A. HERMAN, Executive Secretary of the National Assoc. of Artificial Breeders, was the principal speaker. His topic was "The opportunity and responsibility in the artificial breeding of dairy cattle."

Oklahoma:

MILFORD BONNER, a graduate of Mississippi State College in 1951, has resumed his graduate work after a tour of duty in Korea.

Michigan State:

The second annual Dairy Engineering Conference will be held March 3 and 4, 1954, at the Kellogg Foundation Building.

Tennessee:

The American Dairy Assoc. of Tennessee has given a $200 award to each of four juniors and three seniors majoring in dairying.

HUGH O. JAYNES was the 1953 winner of the Purina Bowl awarded each year to the most worthy student in dairying. He was also given

the $300 Borden award for having the highest scholastic average in the College of Agriculture at the beginning of his senior year. At present Mr. Jaynes is a research assistant in the department.

Rhode Island:

CLIFFORD J. KOSIKOWSKY, a graduate of the Univ. of Connecticut, has been appointed an instructor in the Department of Animal and Dairy Husbandry. He will manage the dairy plant and teach the manufacturing courses.

JOHN BUCHANAN, who formerly held the position, has purchased a poultry farm in New Hampshire.

Illinois:

The annual Dairy Technology alumni reunion was held October 17. Lunch was served at the Dairy Manufactures Building for 175 alumni and their friends. After the Illinois football victory over Minnesota, the group again gathered at the building for coffee and doughnuts.

Cooperative Graduate Summer Sessions in Statistics

Beginning in 1954, North Carolina State College, the Univ. of Florida, Virginia Polytechnic Institute, and the Southern Regional Educational Board will jointly sponsor cooperative Graduate Summer Sessions in Statistics.

The first session will be conducted by a distinguished faculty at Virginia Polytechnic Institute in the summer of 1954. Additional summer sessions are tentatively planned for North Carolina State College and the Univ. of Florida in the two following years. Subsequent sessions will be rotated among these or other institutions throughout the South.

The summer sessions are designed to carry out a recommendation of the Southern Regional Education Board's Commission on Statistics, on which the three institutions initiating the program are represented. They will be of particular interest to (1) research and professional workers who want intensive instruction in basic statistical concepts and who wish to learn modern statistical methodology; (2) teachers of elementary statistical courses who want some formal training in modern statistics; (3) prospective candidates for graduate degrees in statistics; (4) graduate students in other fields who desire supporting work in statistics; and (5) professional statisticians who wish to keep informed of advanced specialized theory and methods.

Each of the summer sessions will last 6 weeks and each course will carry 3 semester hours of graduate credit, with a maximum of 6 semester hour credits earned in one summer. The courses are arranged to enable the person to take consecutive work in successive summers. The summer work in statistics may be applied at any one of the cooperating institutions in partial fulfillment of the requirements for a Master's degree. The catalog requirements for the degree must be met at the degree-granting institutions. Each Doctoral candidate should consult with the institution from which he desires to obtain the degree regarding the applicability of the summer courses in statistics.

During the first session PROFESSOR MAURICE KENDALL of the Univ. of London will give a course in Multi-variate Analysis, and DR. RALPH COMSTOCK of North Carolina State College will give one in Quantitative Genetics.

The staff of the Virginia Polytechnic Institute's Department of Statistics will offer such courses as Probability and Inference, Analysis of Variance, Statistical Methods, Engineering Statistics, Education Statistics, Rank Order Statistics and the Theory of Sequential Methods.

The department includes R. A. BRADLEY, D. B. DUNCAN, M. C. K. TWEEDIE, P. M. SOMERVILLE and BOYD HARSHBARGER. In addition, other outstanding statistical scholars will direct special afternoon seminars. The agricultural, science and engineering divisions of the College will make available advanced courses for students who wish to supplement their work in statistics.

The fee for the Virginia Polytechnic Institute session is $30.00. Board, room, post office box and laundry for the entire session may be had for $76.40. The session will run from June 9 through July 17, 1954.

Inquiries should be addressed to BOYD HARSHBARGER, Head, Department of Statistics, Virginia Polytechnic Institute, Blacksburg, Virginia.

Wells Joins Pet Staff

CLAUDE B. WELLS, JR. has become a member of the general office staff, Pet Milk Co., Johnson City, Tenn. Wells graduated from North Carolina State College with a major in dairy manufacturing and took his M.S. degree at Virginia Polytechnic Institute. He has had positions with the Evaporated Milk Assoc., Rochester Dairy, Rochester, Minn., The Borden Co., and the General Equipment Manufacturing and Sales Co., Indianapolis, Ind.

E. B. Hart Honored

A portrait of the late E. B. HART, pioneer biochemist at the Univ. of Wisconsin, has been hung in the Pioneer Room of the Dairy Shrine Club at Waterloo, Iowa. He is the 52nd dairy pioneer to be so honored by the Club.

Professor Hart became chairman of the Department of Agricultural Chemistry at the Univ. of Wisconsin in 1906. He retired in 1944 and died March 12 of this year. It was under his direction that vitamin A was discovered and its function established. His laboratory helped to unfold the story of the vitamin B group.

Other discoveries included the demonstration of the relationship between carotene and vitamin A; the irradiation of foods to produce vitamin D activity; proof that pellagra could be cured by niacin, that copper and iron are needed for the production of hemoglobin, and that the coagulation of evaporated milk could be prevented by a proper balance of the salts in the milk before sterilization.

Professor Hart was a recipient of the Borden award in 1941 and was a member of the National Academy of Science. He received an honorary doctor of science degree from the Univ. of Wisconsin in 1949.

Michigan State College School of Graduate Studies

The School of Graduate Studies, Michigan State College, offers the following appointments to qualified students, predoctoral and postdoctoral. Inquiry should be directed to the Dean, School of Graduate Studies, unless other directions are given.

Graduate assistantships. Stipends range from $1,000 to $1,400, with a few at higher values, depending upon the nature and extent of service required. Direct inquiries to the appropriate department heads.

Tuition scholarships. These appointments carry remission of fees and, in the case of some foreign students, an additional award of $75.00 per quarter.

Predoctoral fellowships. About twenty such appointments are made each year. Stipends range from $500 to $1,200, and in most, but not all, cases fees are waived. No service to the College is required.

Postdoctoral fellowships. One of these, carrying a stipend of $3,000, is awarded each year for research in a field for which the college has appropriate facilities.

Annual Meeting of the Eastern Division A.D.S.A.

The annual meeting of the Eastern Division, A.D.S.A., was held at West Springfield, Mass., Sept. 20-21, in conjunction with the Eastern States Exposition. The following papers were presented at the production section:

Antibiotics for Dairy Calves—CHARLES EDGERLY, Univ. of Maine.

Predicting the Transmitting Ability of Dairy Sires—H. C. DICKEY, Univ. of Maine.

Improving the Utility of Poor Quality Hay—JOHN ARCHIBALD, Univ. of Massachusetts.

Estimates of Liver Stores of Carotenoids and Vitamin A in the Dairy Calf—C. M. DEMBICZAK, J. E. ROUSSEAU, Jr., L. A. MOORE, GEOFFREY BEALL, and H. D. EATON, Univ. of Connecticut and U.S.D.A.

Rate of Carotenoid Loss in Artificially Dehydrated Alfalfa under Farm Storage—K. L. DOLGE, H. D. EATON, J. E. ROUSSEAU, Jr., and L. A. MOORE, Univ. of Connecticut and U.S.D.A.

The Value of Coarse and Fine Concentrates for Dairy Cows—H. A. KEENER and N. F. COLOVOS, Univ. of New Hampshire.

Low Temperature Storage of Bull Semen—JACK SAROFF, Rutgers Univ.

At the Manufacturing Section the following papers were presented:

The effects on Cream Quality of Separating Cold Pasteurized Milk—L. B. GLAZIER, A. H. MANKE, and L. R. DOWD, Univ. of Conecticut.

Tentative Identification of Malty Aroma Substance from Milk Cultures of *Streptococcus Lactic* var. *Maltigenes*—H. JACKSON and M. E. MORGAN, Univ. of Connecticut.

Bacteria which Grow in Milk During Refrigerated Storage: A Review of the Literature—H. V. ATHERTON, Univ. of Vermont.

An Evaluation of Thermal Effectiveness of the Pasteurizing Process in Commercially Operated High Temperature Short Time Milk Pasteurizers—JOHN L. FOLEY and D. J. HANKINSON, Univ. of Massachusetts.

The Eastern States Intercollegiate Dairy Cattle and Dairy Products Judging contests were held on September 21-22. Cornell Univ. ranked first in dairy cattle and Univ. of Conn. took high honors in dairy products.

Officers elected for the coming year were: Chairman, RICHARD C. FOLEY, Univ. of Massachusetts; Vice-chairman, ALEC BRADFIELD, Univ. of Vermont; Secretary-Treasurer, GEORGE W. TRIMBERGER, Cornell Univ.

Ohio State Wins Contest at Waterloo

The dairy cattle judging team from Ohio State won first place in the judging of all breeds at the 1953 National Dairy Cattle Congress at Waterloo, Iowa. The Cornell team was second, followed in order by Purdue, Maryland, and Penn. State. The winning individual was CHARLES GRIMES of Ohio State. For the different breeds the teams placing first were: Ayrshires, Texas A. & M.; Brown Swiss, Purdue; Guernsey, Cornell; Holstein, Cornell; and Jersey, Kentucky.

Carnation Opens New Laboratory

The formal opening of the Carnation Research Laboratory at Van Nuys, Calif., was held October 8. E. B. OBERG is Director of Research.

The Technology of Modern Sanitation

A Guest Editorial

The developments in the field of modern cleaning and sanitizing are many and varied. In fact, they have come on so rapidly and there have been so many of them that the state of confusion which exists today in the minds of many people is greater than ever before.

Years ago, cleaning problems were simple and the technology of cleaning was simple. There were few basic raw materials available and their uses were limited. Fundamentally, these few basic commodities consisted of such old standbys as caustic soda, metasilicate of soda, soda ash and its several derivatives, and trisodiumphosphate. Cleaning chemistry, therefore, was largely limited to the values, uses, and applications of these materials, or various combinations of them.

But now all of this has been changed. The development of a number of new and important groups of cleaners and sanitizers in recent years has made possible new approaches to cleaning problems and has brought about a new standard for sanitation. Today it is possible to state categorically that surfaces may be cleaned by proper application of balanced cleaners to a state where they are chemically, physically, and bacteriologically clean. The advent of such important groups of materials as the polyphosphates, the application of the wetting agents to the cleaning field, the development of the organic cleaners, and more recently the development and application of the chelates—all represent forward steps in the over-all development of cleaning technology. However, even these accomplishments are simple compared with some of the work which is now going forward. The application and utilization of enzymes in cleaning, the setting up of electrical fields, the use of radio isotopes, and many other new techniques are well past the experimental stage. There is no question but that many of these recent developments will make it possible to establish completely new standards for cleanliness and sanitation.

C. B. Shogren

What has been said of the cleaning field is likewise true of the sanitizers themselves. The advent of the quaternary ammonium compounds a few years ago was hailed by many as a completely new and revolutionary approach which would make sanitizers, such as chlorine, the hypochlorites and other commonly used sanitizers, completely out of date. This has not proved true, however, and we believe it is possible to state that the quaternary ammonium compounds, while exceedingly useful, will probably find their ultimate usefulness as a component of the cleaners bearing germicidal properties.

Another new advancement which holds considerable interest to many research workers in this field is the development of the iodophors. These are fundamentally complex compounds, combining the highly desirable bactericidal qualities of iodine with certain of the nonionic wetting agents. They form a completely new complex which is exceedingly active germicidally. While work in this field is incomplete, no doubt something useful will develop from it.

In summing up, the confusion which exists in the fields of detergency and sanitizers is a natural and logical outgrowth of the tremendous chemical research and advance which has taken place within the past twenty years. No industry can remain static. It is certainly true that in the chemical industry, particularly in the field of chemical detergency, rapid advances have been made. It is unfortunate that everyone does not use common sense to utilize all of these fine advances to the best possible advantage. However, little by little, as people come to understand the real values and properties of some of these newer materials and the methods of applying them in compounds to make cleaning easier and to do the job better, new standards will be set and a higher quality of sanitation will be enjoyed by all food industries.

C. B. Shogren
Klenzade Products Co.
Beloit, Wis.

LETTERS TO THE EDITOR

The Journal of Dairy Science Can Help to Bring About Closer Cooperation Between Universities and the Dairy Industry

Your recent announcement regarding publication of a "Letters to the Editor" column may well be the beginning of a valuable new service to the Association. The Journal's present functions will be augmented by one which could be developed into an instrument for the interchange of ideas on current problems and new developments leading to suggestions of new research areas.

It is becoming increasingly difficult for many university people to keep abreast of all new developments in the dairy industry. Tight teaching and research schedules do not allow for extensive travel and attendance at industrial meetings and conferences, with the result that immediate problems of the industry often are not well understood. It would be helpful if this column of the Journal contained letters from industrial research laboratories pointing out current problems that might lend themselves to university research. Such communication of ideas should lead to closer cooperation and better understanding. Health regulatory and other governmental agencies also should be invited to contribute to this column to enumerate those developments in which they are currently most interested.

During a recent conversation with a member of a health regulatory agency, several new problems were discussed which could not be solved satisfactorily because of a lack of experimental reports on the subject. It is believed that a "Letter to the Editor" of this Journal would have brought a response from research workers who were possibly working on a related problem or who would have been interested to attempt a solution to these particular problems.

A somewhat specific example on how this Journal may be of good service to the industry may be derived from the following experience. Many plant operators have had difficulties with chocolate milk when laboratory reports consistently indicated a positive phosphatase test in spite of the fact that the product may have been heated to 180° F. for as long as 30 minutes. A sample of such a chocolate milk was once examined and found to contain methyl vanillin, which was responsible for the falsely positive phosphatase test. The incident caused considerable embarrassment to the dairy, as well as to the manufacturer of the chocolate syrup. In this case a short "letter to the editor" may have been a satisfactory explanation to the local health regulatory agency involved.

While most contributions to the new column should be voluntary it also may be desirable that the editor invite letters from appropriate laboratories and agencies periodically to insure coverage of current topics of interest.

It is hoped that the "Letters to the Editor" column will serve its purpose well.

J. TOBIAS
Assistant Professor of Dairy Technology
University of Ill.

Ropiness in Mix

Recently a sample of ropy commercial ice cream mix was sent to our Laboratories for bacteriological analysis. An examination of the sample showed it to be contaminated primarily with a yeast and a bacterium; further study indicated the bacterial contaminant to be *Pseudomonas fluorescens*. In view of the fact that this organism is often involved in ropy milk outbreaks, an effort was made to produce this defect in skimmilk culture, but all attempts were unsuccessful.

Since the fulfillment of Koch's postulates is a necessary requisite for determining the causative agent in any type of infection or spoilage problem, it was essential that the ropy defect be reproduced using the suspected culture. Ice cream mix was then tried as a culture medium. After 2 days incubation at 20° C. a ropy condition was observed; no ropiness was observed in the mix inoculated with the yeast isolate. It would appear from this that the use of ice cream mix as a culture medium for detecting ropy contamination might be desirable in ice cream operations. Line samples, water supplies, etc. could easily be screened for ropy contamination in this way.

The failure of some *Pseudomonas* strains to produce ropiness in skimmilk might also account for the many "missed cases" or "unsolved incidents" involving a ropy defect.

O. W. KAUFMANN
Assistant Professor of Microbiology
University of Ill.

The Curriculum Problem

I am writing you concerning the Curriculum Committee's procedure to secure information concerning the revising of the curriculums in the colleges.

I believe that they would secure better information if they would contact men and workers in the different industries. About ten years ago, we made a survey where each department contacted owners, managers, superintendents and even the workers in the different departments, and from this information we revised our present courses and also added or deleted courses that were not in accordance with the survey.

I thought it might not be too late to suggest this plan, as I understand the work of these committees has not been closed.

E. M. MYERS
Head of Dairy Department
State University of New York
Alfred, N. Y.

The "Rainbow" on Stainless Steel

Several months ago we had the opportunity to examine some sections of stainless steel pipe used in your milk processing equipment. These sections showed on the inside of the pipe a slight coloration indicative of the interference colors from a very thin film of some kind, much the same as an extremely thin layer of oil on water. We undertook to determine the nature of this adherent colored film, particularly to determine whether it was produced from some interaction with milk.

It was clearly established that these colored areas were simply metal oxides and were not due to any organic deposit or to any inorganic deposit from detergents or otherwise. While stainless steel is supposed to be resistant to oxidation and corrosion as an alloy of iron, chromium, and nickel, it is still true that the actual product itself represents formation of extremely thin, adherent, and residual oxide films. The interference colors, or course, indicate the extreme thinness of the layers with respect to the wave length of visible light. If this film should become thicker, these interference colors would then disappear. The resulting thicker film is largely transparent and invisible.

It is certainly logical to conclude that the formation of these colored areas does not represent a serious condition and cannot in any way contaminate milk. Just why the colored areas appear in streaks and in localized areas is difficult to determine, although some X-ray diffraction patterns would indicate that there is a nonuniform strain and grain size distribution in these tubes and the color seems to appear in a very sensitive way over the areas which are most highly strained, that is, junction points, bends, etc. It is obvious that some type of oxidation is involved, which means that air must get at the metallic surfaces and the oxidation is probably catalyzed at the high temperatures and probably by constituents in the milk along with the indications of the nonuniform metallic texture.

I believe that this is a very interesting and perhaps new observation made possible only by the powerful technique of electron diffraction. These films are far too thin to give indications of composition and structure by X-rays which are far more penetrating.

I hope that these remarks will be helpful to you in passing this information on to other interested people.

G. L. CLARK
Professor of Chemistry
University of Ill.

A Proposal to the President of A.D.S.A.

The order of authorship upon scientific articles has always been a source of confusion to me. There do not appear to be any standards established by the scientific journals except the rather vague custom that the authors be arranged in the order of decreasing magnitude of their contribution to the paper. This rather doubtful procedure has little to recommend it, provided that the list of authors includes only those closely associated with the study reported and does not include honorary authorships. Under these conditions, the paper could not have been done without the contribution of the various authors, the relative value of which is usually extremely difficult to measure.

I would like to propose a different procedure for use in the *Journal of Dairy Science*. The order of authorship should be related to the type of contribution rather than its magnitude. In recent years the authorship of a paper usually included the following: the research director or project leader responsible for the over-all planning and continuity of the study, the laboratory worker who performs the majority of the detailed experimentation and, finally, others who make more specialized contributions of sufficient magnitude to be recognized. While I do not advocate any particular order of the above contributions, the establishment of a fixed order on the basis suggested would dispel the existing confusion. One could tell from the order who was the project director so that inquiries concerning reprints and project policies and plans would be addressed to him, and who was the laboratory worker from whom information concerning the detail of the work could best be obtained. Thus the order of authorship would serve some useful purpose other than that of indicating some questionable magnitude of contribution.

Therefore, I would recommend that it be proposed to the President of the American Dairy Science Association that a committee be selected to consider this problem in order that a definite policy concerning the authorship of papers may be established for the *Journal of Dairy Science*. Such a policy might in turn encourage other journals to do likewise.

R. MCL. WHITNEY
Associate Professor of Dairy Technology
Univ. of Ill.

A Former Dairy Technology Student Speaks

As a former student in Dairy Technology in one of the Big Ten Universities I was interested in Professor Gould's recent editorial in the *Journal of Dairy Science*. Here is why I think more young men do not go into the field of Dairy Manufacture or stay after they start.

1. Too much hard physical work on hard floors in an uncomfortable temperature and humidity.
2. Hours too long, 7 days a week in some cases, and pay too low—especially for

laboratory workers and quality control men.
3. Rubber boots hard on feet and legs.
4. Too many early-in-the-morning and late-into-the-night hours.
5. Credit for growth of business given to truck drivers and salesmen and none to plant workers and supervisors.
6. No rest periods during day.
7. Too many times plant worker has to eat cold lunch in an unattractive room.
8. Too many times there is no comfortable lounge room equipped with radio or television or supplied with good magazines—especially trade journals and research publications such as the *Journal of Dairy Science*.
9. No personnel director to look after employees' interests in most of the smaller plants.
10. Lack of suitable sick leaves, retirement plans, health insurance, and encouragement for men to participate in outside activities, such as church, Boy Scouts, luncheon clubs.
11. Failure of company to send men to trade association meetings, dairy shows, and dairy technology society meetings (at company expense).
12. Not enough attention given to the importance of the plant laboratory and the contributions of technically trained workers in the organization.

I realize that the above points do not apply to all organizations or dairy plants today, but they apply to a large enough number to cause many a man trained in dairy technology to turn to another occupation after a few years of disappointment as to his future in the chosen field and certainly explain why many good young men do not enter the field today.

DALE FAULKNER
Tuscon, Arizona

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

WHAT IS NEW IN THE NUTRITIVE VALUE OF MILK[1]

C. A. Elvehjem

Department of Biochemistry
University of Wisconsin, Madison

Although milk has been used as an important food for centuries, the explanations of its exceptional nutritional values have been elucidated only within the present century. Modern research techniques have been used to determine the composition of milk and the importance of each of the components in diets for optimum nutrition. Many facts have been established, but additional findings are still being made.

Milk important source of vitamin B_{12}—the antipernicious anemia factor

One of the newest findings undoubtedly is the fact that cow's milk contains 3 to 4 γ of vitamin B_{12} per liter. One might assume that the presence of such minute quantities of a chemical compound, even if it be a vitamin, would have little significance. However, it is important to emphasize that a human being suffering from pernicious anemia will respond to the administration of 1 γ of vitamin B_{12}. Therefore, 3 to 4 γ per liter of milk is of importance and renders milk an important food as far as this vitamin is concerned. It has taken many years to clearly identify vitamin B_{12} as the antipernicious anemia factor and to develop methods which will determine accurately the amount of this vitamin in foods.

C. A. Elvehjem

Thirty years ago, when it was shown that weanling rats would develop anemia even when milk was fortified with iron, attempts were made to find a factor which would supplement the iron for hemoglobin formation. At that time liver extracts, which had been prepared for the treatment of pernicious anemia, were found to be active. However, it was later shown that these materials could be ashed without destroying their activity and therefore the factor was separate and distinct from the so-called pernicious anemia factor which was organic in nature. Later the active material was shown to be copper and that when both iron and copper were added to milk, normal blood formation took place. It was also shown that milk was low in manganese but that when cow's milk was supplemented with the three mineral elements, iron, copper and manganese, excellent growth was obtained in several species of animals for extended periods of time and good reproduction was obtained in both rats and dogs. At that time, then, it was evident that milk could not be very deficient in the antipernicious anemia factor, although one sometimes wondered if experimental animals needed this factor. Today we know why it was so difficult to produce a deficiency of the antipernicious anemia factor—it is needed in such minute quantities.

Cow's milk superior to goat's milk in vitamin B_{12} Content

In contrast to cow's milk, goat's milk often has been associated with severe anemia in the human infant. Thus the term "goat's milk anemia" gained common usage. Since liver, liver extracts, and yeast were found to prevent this condition, György in 1934 suggested that goat's milk might be deficient in Castle's "extrinsic factor" or part of the antipernicious anemia factor. Studies in our own laboratory showed that mineralized goat's milk and mineralized cow's milk were of equal activity in preventing and curing anemia in young rats. However, the growth rate of animals fed the goat's milk diet was inferior to that obtained with cow's milk.

Little attention was given to this observation for about 15 years, but during that time extensive studies were made on the identification and isolation of vitamin B_{12}. Today vitamin B_{12} is a well-known compound, and it is known to contain cobalt. In fact, the vitamin B_{12} which is found in beef liver is undoubtedly produced in the rumen of the cow when the diet contains adequate amounts of cobalt. Using the microbiological assay for both vitamin B_{12} and folic acid, it has been found that cow's milk contains 3 to 4 γ of vitamin B_{12} and 1 to 3 γ of folic acid per liter. Since there are certain forms of vitamin B_{12} which are active for micro-

[1] A more detailed discussion of some of these facts will appear in a forthcoming issue of the Quarterly Review of Pediatrics.

organisms and not active for the animal, it was important to demonstrate that the vitamin B_{12} present in milk is active for animals. This was possible by comparing the growth of rats on mineralized cow's milk and mineralized goat's milk since goat's milk contains only about 0.2 γ of vitamin B_{12} per liter. The growth of the animals on goat's milk was markedly inferior to that obtained on cow's milk. Analysis of the livers of these animals indicated that the vitamin B_{12} content of the livers from rats receiving goat's milk was considerably less than those receiving cow's milk. When goat's milk was fortified with vitamin B_{12} to a level equivalent to that in cow's milk, normal growth was obtained and the vitamin B_{12} content of the livers was comparable to that obtained when cow's milk was fed.

Cow's milk contains 4 to 6 times as much vitamin B_{12} as does human milk

This work clearly demonstrates that the vitamin B_{12} in milk, as measured by microbiological assay can be utilized by the normal animal. It is interesting to point out that little or no anemia occurred in the rats on goat's milk, although the intake of vitamin B_{12} was so low as to reduce growth. It appears, then, that in this species the amount of vitamin B_{12} required for hemoglobin formation may be less than that which is necessary for rapid growth. In those areas of the world where goat's milk is used in infant feeding, some additional source of vitamin B_{12} should be supplied. On the other hand when cow's milk is used there appears to be no reason for supplying extra amounts, since cow's milk is considerably higher in vitamin B_{12} than is human milk. Human milk contains about 0.5 γ of vitamin B_{12} per liter.

FIG. 1. Both rats were placed on whole milk at weaning. The animal on the left received the whole milk alone and the animal on the right received the whole milk fortified with iron, copper and manganese. The animals were 51 days old. The one on the left weighed 60 g. and the one on the right 120 g.

Milk not a rich source of vitamin B_{12}

If we return for just a moment to folic acid, we find that the folic acid content of cow's milk and goat's milk is about equal. It is interesting that when we add folic acid alone to goat's milk, some improvement in growth is obtained, which indicates that folic acid has a sparing effect on vitamin B_{12} when it is present in suboptimal amounts. Cow's milk then is not a rich source of folic acid, but on a diet consisting entirely of mineralized milk no improvement is obtained by adding extra amounts of folic acid. We had an interesting experience when mineralized cow's milk was fed to young mink; these animals developed very well on such a diet although after about a year some reduction in growth occurred, which could be overcome by adding extra amounts of folic acid. Thus, milk is probably on the borderline as a source of folic acid for this species, which is known to have a high folic acid requirement. In a mixed diet for humans other foods would amply supplement the folic acid intake. Thus in infant feeding the use of vegetables and other products early in the infant's diet adequately insures an ample intake of this vitamin.

New angles to pasteurization effects

The fact that mineralized whole milk diets are satisfactory for rapidly growing animals gives us further opportunity to study the possible deleterious effects of pasteurization or heat treatment of milk. Twenty years ago we showed that rats grew as well on pasteurized mineralized milk as on raw mineralized milk. Such experiments have been repeated recently, and we have been unable to detect any deleterious effects due to pasteurization when rate of growth was used as the criterion. In these studies a rather interesting observation was made when the livers of these experimental animals were analyzed for fat. A normal liver contains 4 to 5% fat on the wet weight basis. The livers from animals receiving the milk diets contained 6 to 8% fat. The level of this fat can be reduced by feeding milk with a lower fat content. Other studies indicate that the lactose in milk may be partially responsible for the tendency for fat to accumulate in the liver. The interesting point, however, is the fact that when the milk is pasteurized or heated under any conditions the level of fat tends to be slightly lower. This suggests that heating makes the proteins in milk more available since it has been shown that certain amino acids,

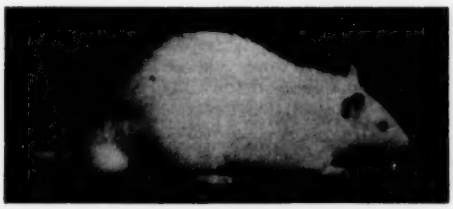

FIG. 2. This rat represents the fifth generation reared on mineralized whole milk. The photograph was taken at 15 weeks of age. Weight 284 g.

especially threonine, are effective in maintaining a normal level of fat in the liver. In any case, no deleterious effects were evident as a result of heat treatment. We must not overlook the fact that there may be some loss in vitamin C and vitamin B_1 and that pasteurization does reduce the folic acid content to a slight extent, although it has no effect on the vitamin B_{12} content.

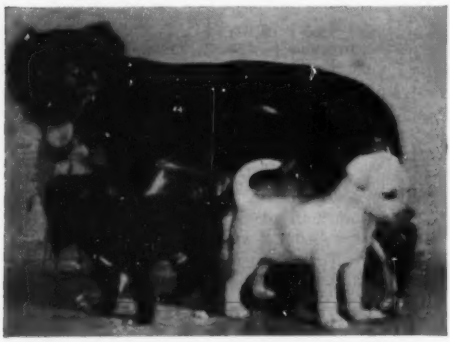

FIG. 3. The female dog was raised from weaning on pasteurized whole milk fortified with iron, copper, manganese and cod liver oil. The pups represent her first litter at weaning time. They were 35 days of age—both males—and each weighed about 1 kilo. The dam was 18 months of age and weighed 5.6 kilos.

New growth factors may be present in milk

Since growing rats do so well on mineralized whole milk, it is difficult to study additional factors which may or may not be present in milk in adequate amounts. The guinea pig may serve as a valuable experimental animal for this purpose. Guinea pigs do not grow very well on mineralized cow's milk, but some improvement can be obtained by adding various types of roughage. There appears to be great variation in the response obtained with individual animals. Some do remarkably well, and others grow poorly. These results tend to indicate that the variation is due to changes in the intestinal flora. The guinea pig is not adapted to a milk diet, since the guinea pig suckles for only a very short time and begins to eat a mixed diet very early. Nevertheless, by following through these studies, further information may be gained regarding the nutritional significance of new factors in milk.

György and his coworkers have been studying the effect of certain factors in milk on the growth of microorganisms in the intestinal tract. It is well known that infants fed human milk harbor a larger number of *Lactobacillus bifidus* organisms in the tract than do infants fed on cow's milk. These workers found a factor present in human milk which was necessary for the growth of certain strains of *Lactobacillus bifidus*. Cow's milk appears to be very low in this factor, and human milk and milk from certain other species contains variable amounts of this substance. One would expect that cow's milk would fail to produce an optimum response in animals if this factor were present in low amounts. We have tried adding mucin as a source of this factor to our mineralized cow's milk diets for rats but have obtained no response.

It is important that cow's milk be supplemented with vitamin C for infant feeding

This indicates that future work will have to depend upon the use of a variety of experimental animals, and perhaps greater emphasis will be given to changes taking place in the flora of the intestinal tract. Dr. May and his coworkers have used the monkey to study the possible limitations in milk diets. They have been interested especially in the relation of scurvy to megaloblastic anemia. It appears from their studies that during scurvy there develops an increased requirement for folic acid compounds, and milk diets then do not supply sufficient amounts of this vitamin. In other words, milk may need to be supplemented with folic acid when certain other stresses are placed on the animal. This may be of real importance in infant feeding and indicates the importance of supplementing infant diets with vitamin C, not only to prevent symptoms of scurvy but to prevent increased requirement of other factors, such as folic acid.

Although there is no indication that pasteurization of milk reduces its nutritional quality to any significant extent, we should keep in mind that milk finds its way into many processed foods. During this processing certain changes may occur which alter nutritional requirements. There are some recent observations which indicate that certain processed infant foods require larger amounts of vitamin B_6 for the normal development of the infant. This increased requirement is at present little understood, but there may be numerous relationships of this kind.

Further studies needed

In conclusion, one can perhaps say that most of the important nutrients in milk have now been identified, although there may be a few factors which have been completely overlooked. The experience with vitamin B_{12} clearly indicates that we must deal with substances that are present in trace quantities. However, in the future our biggest problem will be connected with methods of using milk in human diets and how these milk products may be introduced to yield optimum responses.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

960. An intensive study of postvaccination responses in groups of calves vaccinated intracutaneously and subcutaneously with strain *19* Brucella Abortus vaccine. C. M. COTTON, Univ. of Md., College Park. Am. J. Vet. Research, **14**, 52: 337-342. 1953.

Two comparable groups of 108 calves each were used in this field experiment. Intracutaneous vaccination with 0.2 ml. and subcutaneous vaccination with 0.5 ml. of vaccine was made at 4 to 7 mo. of age. Blood samples were examined before vaccination and for 14 daily tests afterwards, and monthly tests up to 24 mo. The intracutaneous vaccinations produced higher titers, but the titers were lost before were those from subcutaneous vaccination. After 6 mo. all except 3 of the intracutaneously vaccinated had negative blood agglutination titers, but only 64.8% of the subcutaneously vaccinated calves were negative. Sixteen of the calves had nursed positive cows and showed positive opsonocytophagic responses. These calves did not respond to the vaccinations with a marked titer rise and quickly lost the low titer they developed. Also, 4 of them acquired brucellosis after natural exposure at 2½ yrs. of age. The blood lymphocytes increased by the 4th day and remained high through 2 mo., paralleling the highest agglutinin and opsonin activity.

E. W. Swanson

961. Serology of brucellosis in rural Indiana. S. R. DAMON, Ind. State Board of Health, C. R. DONHAM and L. M. HUTCHINGS, Purdue Univ., Lafayette, B. T. SIMMS. U.S.D.A. and J. H. STEELE, Pub. Health Service. Pub. Health Reports, **68**, 6: 563-571. 1953.

Serological methods were used to determine the prevalence of Brucella agglutinins among farm famiiles and livestock in 9 rural townships in Indiana. Of the 2,622 persons 14 yr. of age and older who were tested, 1.8% reacted positively to some degree. The % of positive reactors was similar in each township. A total of 930 farms were surveyed and 260 had livestock reactors. Not all the livestock in the 9 townships were tested. Approx. 6% of the 13,373 animals tested were reactors. This included 0.7% of the sheep, 2.5% of the horses and mules, 4.0% of the swine and 6.9% of the cattle tested. When the results of testing those cattle previously vaccinated (10%) were omitted from the data, the number of reactors dropped to 5%. The % of reactor animals varied considerably in the various townships. D. D. Deane

962. Anthrax in the United States. J. H. STEELE, Pub. Health Service, Atlanta, Ga., and R. J. HELVIG, Bur. of State Services. Pub. Health Reports, **68**, 6: 616-623. 1953.

The distribution of human anthrax cases reported during 1945-1951 is presented. In 1951, 60 cases were reported; 45 cases in the northeastern states, 26 of these in Pa. alone. These cases were attributed to occupational exposure involving mostly veterinarians and farmers who came in contact with infected animals. Some cases of infected animals were traced to contaminated bone meal. No cases of human anthrax caused by ingestion of contaminated milk or meat have been reported in the U. S. in recent years.

Anthrax control procedures recommended by the Pub. Health Service are given. The procedures that should be followed in handling milk from a farm where anthrax has been found may not be the same in all cases. Unduly stringent quarantine measures may discourage the reporting of such cases by farmers and the exclusion of the entire milk supply may present a serious economic problem for the dairymen.

D. D. Deane

963. Effect of certain drugs alone and in combination on micrococci isolated from the bovine udder. G. T. EDDS, M. H. ROEPKE, and R. N. BIETER. Fort Dodge Lab., Fort Dodge, IA. Am. J. Vet. Research, **14** 52: 355-361. 1953.

Ten strains of *micrococcus pyogenes var. aureus* which had been isolated from cows with acute and chronic mastitis were used as test organisms to determine their susceptibility in tryptose-phosphate broth to added antibiotics and sulfonamides. Growth of all of the cultures was inhibited by penicillin, streptomycin, aureomycin, or terramycin alone at 5 U or μg. per ml. Penicillin was slightly more efficient on most of the cultures, requiring 0.5 U or less to inhibit growth of all except one strain. Sulfisoxazole up to 500 μg. per ml. was not effective in inhibiting growth of the micrococci. When penicillin

was combined with 50 μg. sulfisoxazole per ml. growth of all strains was inhibited by 0.02 U per ml. or less. A similar enhancement of the killing power of aureomycin by mixing with sulfisoxazole was noted, but little change was effected by mixing with streptomycin or terramycin. When the antibiotics were combined in various groups of two, increased effectiveness was secured from penicillin plus streptomycin, streptomycin plus terramycin, and terramycin plus aureomycin; but little change resulted from mixtures of penicillin plus aureomycin or penicillin plus terramycin. The effective mixtures demonstrated here were suggested for use in clinical work. E. W. Swanson

964. A theoretical discussion of ruminant ketosis. R. B. JOHNSON, Univ. of Md., College Park, Md. Am. J. Vet. Research, **14**, 52: 366-375. 1953.

A review and discussion is presented to show that ketosis develops in ruminants as a result of changes in the rumen fermentation which result in wide ratios of propionic acid to the other short chain fatty acids. This overburdens the catabolic system requiring glucose causing a lower blood sugar level. At the same time fat catabolism is increased to furnish energy and this contributes to more ketone bodies. It is proposed that the ideal treatment for ketosis would be to restore the proper balance of short chain fatty acids in the rumen either by chemical feeding or by diet alterations which will produce the desired rumen fermentations.
E. W. Swanson

965. Research uncovers additional information on the cause of and treatment for ketosis (acetonemia). ANON. Md. Agr. Expt. Sta., College Park. 65th Ann. Rept., p. 40. 1951-52.

Most cases of ketosis recovered quickly and without relapse after a single injection of either corticotropin (ACTH) or cortisone (1½ g. of cortisone or 300 Armour U of ACTH). Several cows which had collapsed completely and would not respond to any other known treatment responded satisfactorily to these materials.
R. W. Hunt

966. The pathology of experimental hypovitaminosis A in young dairy animals. C. F. HELMBOLDT, E. J. JUNGHERR, H. D. EATON, and L. A. MOORE, Storrs Agr. Expt. Sta., Conn. Am. J. Vet. Research, **14**, 52: 343-354. 1953.

Since most previous reports of hypovitaminosis A pathology in cattle very likely included extraneous details, this study was designed to determine which of the pathological signs were specific for the condition. Calves were rapidly depleted of vitamin A after 105 d. by a ration which still allowed normal weight gains. Some of the depleted calves were treated with carotene, vitamin A, or ascorbic acid. Consistent signs of vitamin A deficiency were diarrhea, incoordination, increased spinal fluid pressure and squamous metaplasia of the interlobular and main ducts of the parotid glands. Some of the depleted animals also exhibited focal necrotic hepatitis and pneumonia detected postmortem (not clinical). Only one cystic pituitary gland was found, and the cells of the pituitary were not notably altered. Changes in the adrenal glands, kidneys, testes, thyroids, and intestines were not in relation to the hypovitaminosis A. The article has a concise review of vitamin A deficiency symptoms and is illustrated with clear photomicrographs.
E. W. Swanson

BUTTER

967. Production of graded butter in Switzerland. F. LANG, Commonwealth Bur. Dairy Sci., Reading, Engl. Dairy Ind., **18**: 587-590. 1953. (Chem. Abstr., **47**: 10146b. 1953.)
S. Patton

968. Coloring material in annatto-butter color. S. IVERSEN and J. LAM, Univ. Copenhagen, Den. Z. Lebensm.-Untersuch. u.-Forsch., **97**: 1-7. 1953. (Chem. Abstr., **47**: 10146c. 1953.) S. Patton

969. Buttermaking. A. I. ZHELTAKOV. U.S.S.R. 77, 182, Dec. 31, 1949. (Chem. Abstr., **47**: 10150g. 1953.) S. Patton

970. Natural butter. Nederlandsche Organisatie voor Toegepast-Natuurwetenschappelijk Onderzoek ten behoeve van de Boeding (FREDERICK D. TOLLENAAR, inventor). Dutch 71,606, Feb. 16, 1953. (Chem. Abstr., **47**: 9522e. 1953.) S. Patton

CHEESE

971. Bydrage over het bestryden van schimmels op de kaaskorst (Investigations to prevent mold development on cheese rind). H. HENDRICKX and A. DE VLEESCHAUWER. Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent, II: 669-677. 1952.

After salting, cheeses were treated with soln. of sodium-nipagin (15%), nipacombin A (10, 15 and 20%), and vitamin K_3 (20, 100, and 300 p.p.m.) and stored at 10° C. with a humidity of 90-92%. Cheeses treated either with sodium-nipagin or nipacombin A showed very little mold after 6 wk. Vitamin K_3 even in the highest concn. did not prevent mold growth.
W. C. van der Zant

972. Cheese making apparatus. R. MIOLLIS (assignor to The Borden Co.). U. S. Patent 2,654,151. 10 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 71. 1953.

A device for removing curd from a cheese vat, consisting of a movable platform within the vat and a scraper which pushes the curd up and onto the raised platform. R. Whitaker

973. The early history of cheesemaking. E. H. MARTH, Univ. of Wis., Madison. Milk Products J., **44**, 10: 30. 1953.

The history of cheesemaking from the time of the early Greek Empire, until introduction into Wisconsin is presented. The resume is well done and makes for interesting and informative reading. J. J. Janzen

974. A study on the effect of salt as an inhibitor of gas formation in Damietta cheese. G. M. SADEK and A. A. HASSAN, Fac. Agr., Shegin El-Kom, Egypt. Dairy Ind., 18: 313-315. 1953. (Chem. Abstr., **47**: 10146e. 1953.) S. Patton

975. Technical study of the manufacture of Saint-Paulin cheese. A. DE VLEESCHAUWER, J. MOULAERT, and H. HENDRICKX, Rykszuivelstat., Melle, Belg. Mededel Landbouwhogeschool en Opzoekingsstas Staat Gent, 16, 4: 375-405. 1951. (Chem. Abstr., **47**: 9517f. 1953.) S. Patton

976. Cheese. XLVIII. Investigation of the manufacture of cheese with milk acidified by the Elac process. G. SCHWARZ and H. MUMM, Inst. landwirtsch. Technol. Landw. Hochschule, Hohenheim, Ger. Süddeut. Molkerei-Ztg., 71: 1774-1775. 1950. (Chem. Abstr., **47**: 9517g. 1953.) S. Patton

977. Variations in the total dry extract and fat in the interior part of soft cheeses of Camembert type. Consequences for the dairy industry and suppressions of adulterations. J. JACQUET, J. DELACROIX, J. LENOIR, and H. GONDOUIN. Ann. fals. et fraudes, 46: 175-208. 1953. (Chem. Abstr., **47**: 9517h. 1953.) S. Patton

CONDENSED AND DRIED MILKS; BY-PRODUCTS

978. Plus profits in nonfat dry milk. MIKE MICHAEL, Milk Plant Monthly. Milk Plant Monthly, **42**, 9: 26-28. 1953.

The advantages of using nonfat dry milk solids by dairy processors include ease of handling and storing a concentrated product, economies in purchasing, lower labor costs, uniform products with uniform composition, and year-round availability.

High heat powder is made by heating skim milk to a min. of 190° F. and holding it for 20 min. or the equivalent in heat treatment. It is produced for bakery use. Low-heat milk solids are made by heating fluid skim milk to 165° F. for approx. 15 sec. They are used for cottage cheese, buttermilk, ice cream, chocolate milk drinks, etc.

Dry milk production is overlooked by many small plant operators because they fear that their surpluses are too small, or the investment too large to amortize over a reasonable period and show a profit. However, equipment is available for handling surpluses as low as 25,000 lbs. daily.

It is doubtful that there will be large shipments of fluid milk from heavy producing areas into shortage areas. Transportation costs are high, and high priced milk has encouraged milk production in former deficit areas. The probability that in the near future there will be Grade A standards for dry milk products to be sold for use in manufacturing cottage cheese, cultured buttermilk, ice cream, and other items, supports the view that dairy plant usage of powder will continue to grow. It offers greater flexibility of operation to all fluid milk plants. C. J. Babcock

979. Spray dried buttermilk. T. I. HEDRICK, Dairy Branch, PMA, U.S.D.A., Chicago, Ill. Milk Plant Monthly, **42**, 9: 19-20. 1953.

The first concern in the manufacture of dried buttermilk is the production of a high quality product. A good quality product held under favorable conditions should keep more than 2 mo. The cooler the storage temperature and the more moisture resistant the containers, the longer the storage stability. Freedom from added neutralizing compounds is essential for high quality.

There are 2 types of spray dried buttermilk. The more common type is sweet cream buttermilk powder. Acidity of the powder varies from 0.10% to 0.16% (reconstituted). The other type is referred to as high acid, cultured, or ripened buttermilk powder.

Spray dried buttermilk may be used in bakery goods, confections, manufactured dairy products, and as dry milk. C. J. Babcock

980. Dried or evaporated food product, especially milk, and the process of making it. H. A. TOULMIN, JR. (assignor to The Commonwealth Engineering Company of Ohio). U. S. Patent 2,656,276. 5 claims. Oct. 20, 1953. Official Gaz. U. S. Pat. Office, **675**, 3: 769. 1953.

Milk and other liquid food products are dried to form products, which on reconstitution, have substantially the same flavor as the original liquid. The milk first is concentrated by freezing to a slush, removing most of the ice crystals thus formed, then warming the partially concentrated product and finally spray drying to form a dry powder. R. Whitaker

981. Dehydrated foods. Chemistry of browning reactions in model systems. J. E. HODGE, N. Reg. Research Lab., Peoria, Ill. Ag. and Food Chem., **1**, 15: 928-943. 1953.

A review with 201 references. S. Patton

982. Production of calcium lactate and lactic acid from cheese whey. L. A. CAMPBELL. Can. Dairy and Ice Cream J., **32**, 3: 29-31. 1953.

A modified fermentation process is described

for the commercial production of lactic acid and calcium lactate under Canadian conditions. H. Pyenson

983. Hygienic value of kurt (acidophilus-type milk) and rationalization of its production. M. I. Kuchmi and S. K. Sagdieva. Gigiena i Sanit., 1: 52-53. 1953. (Chem. Abstr., 47: 10145b. 1953.) S. Patton

984. Preparation of some forms of dairy products by Kazakh native methods and the results of their chemical analysis. Kh. Makhmudov. Izvest. Akad. Nauk Kazakh. S. S. R. 113, Ser. Biol., 6: 83-89. 1951. (Chem. Abstr., 47: 10145e. 1953.) S. Patton

985. Casein from deteriorated skim-milk powder. V. Subrahmanyan, M. V. Lakshminarayan Rao, M. Swaminathan, and S. Kuppuswamy, Central Food Technol. Research Inst., Mysore, India. Bull. Central Food Technol. Research Inst., 2: 96-99. 1953. (Chem. Abstr., 47: 10145f. 1953.) S. Patton

DAIRY BACTERIOLOGY

986. Numbers and characteristics of lactate utilizing organisms in the rumen of cattle. J. Gutierrez. J. Bacteriol., 66: 123-128. 1953.

Colonies giving a copious black precipitate in a lactate medium to which ferrous sulfate had been added were found to be those of a lactate-utilizing gram-negative curved rod. These bacteria were identified as members of the genus *Desulfovibrio*. They were not present in large numbers, but the colonies appeared within 2 d.

A gram-positive lactate-utilizing rod produced colonies more slowly and was present in numbers ranging from 2.5×10^6 to 7×10^9 per ml. of rumen fluid. These bacteria were present in large numbers on hay and grain and in soil. These organisms are considered to be *Propionibacterium acnes* because of their production of propionic and acetic acids from lactate and their other characteristics. Probably these bacteria are important in production of propionic acid in the rumen, where they apparently grow well.

Veillonella gazogenes appeared in small numbers in rumen contents. F. E. Nelson

987. Rumen microorganisms. Effect of diet and antibiotics on utilization of nonprotein nitrogen. J. M. Prescott, Texas Agr. Expt. Sta., College Station. Ag. and Food Chem., 1, 14: 894-896. 1953.

Because rumen microorganisms are known to be responsible for the utilization of non-protein nitrogen by ruminants, and because of recent interest in the feeding of antibiotics, the effects of several antibiotics on the utilization of urea by rumen bacteria were investigated. Under the conditions of the *in vitro* technique employed, the addition of aureomycin, terramycin, bacitracin, or penicillin caused definite reduction in the utilization of nonprotein nitrogen; the extent of reduction being a function of antibiotic concentration. Rumen liquid from steers which had received a diet containing green roughages possessed a significantly greater ability to utilize nonprotein nitrogen than that of steers fed on grain. These experiments demonstrate that high levels of antibiotics are capable of reducing the utilization of nonprotein nitrogen by rumen microflora *in vitro*, and indicate the desirability of *in vivo* studies before ruminants are fed routinely on a diet containing both urea and antibiotics. S. Patton

988. Studies on the reduction of tetrazolium by lactic acid bacteria. I. Dye reducing activities of different species. H. Laxminarayana and K. K. Iya. Indian J. Dairy Sci., 6, 2: 75-91. 1953.

The dye reducing activities of 15 species of streptococci and 13 species of lactobacilli were studied with 2,3,5 triphenyl tetrazolium bromide. Streptococci, especially *S. lactis* and the enterococci, were more active reducers than lactobacilli. *S. pyogenes, S. cremoris,* and heteroferm. strains of *S. faecalis* were poor reducers. Among the lactobacilli, the *Thermobacterium* group (except *L. acidophilus), L. fermenti,* and heteroferm. strains of *L. plantarum* showed rapid reduction, whereas strains of *L. acidophilus, L. casei, L. plantarum,* and *L. brevis* were poor reducers. *S. thermophilus* showed better reduction in milk cultures whereas *L. dextranicum* was active in broth cultures only. In most instances, species with poor reducing activities showed greater rates of multiplication and acid production in the initial stages than the active reducers. The taxonomic significance of these experiments is discussed.

W. C. van der Zant

989. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. W. L. Kulp, C. Mascoli, and O. Tavshanjian, Bact. Dept., Univ. of Conn., Storrs. Am. J. Pub. Health, 43, 9: 1111-1113. 1953.

The authors have proposed a new agar medium to replace the Endo and EMB media used in the confirmatory test for coliforms in water analysis. The ingredients, in grams per liter of T-7 medium are: Bacto proteose peptone No. 3, 5.0 g.; yeast extract, 3.0 g.; bromthymol blue, 0.25 g.; tergitol-7 (sodium heptadecyl sulfate), 0.1 g.; 2, 3, 5-triphenyl tetrazolium chloride, 0.04 g.; agar, 15 g.; final pH 6.8-7.0 The TTC should be sterilized separately as a strong aqueous solution (autoclaved or filtered) and the correct amount added to the melted and cooled "base" agar before distribution in Petri dishes. Each dish must contain not less than 15 ml. of medium. The medium is stable and poured plates may be kept satisfac-

torily under refrigeration for at least 6 wks. before using. The agar surface is inoculated by streaking in such a manner as to obtain well isolated colonies. The plates should be examined after 12-24 hrs. incubation at 37.5° C. Colony types are more easily differentiated after the shorter time. Colonies of typical *Escherichia coli* are 2-3 mm. in diameter, round, generally entire, very slightly raised, light yellow in color and may have a light, rust-colored center. Typical *Aerobacter aerogenes* colonies are 3-5 mm. in diameter, raised, glistening, yellow, very often slimy and usually have a deep rust-colored center. Both types of colonies may be produced by coliform intermediates. Several of the *Salmonella, Serratia, Proteus, Pseudomonas* species, and *Shigella paradysenteriae* produce irregular, raised, dark-red colonies. *Shigella dysenteriae* failed to grow. The authors believe this medium, since it is inhibitory to Gram-positive organism and does not inhibit coliforms, should be satisfactory for direct enumeration of coliforms in water. D. D. Deane

990. New bacteria spotter. C. P. Burke, Nat. Dairy Res. Lab., Inc., Oakdale, N. Y. Food Eng., **25**, 9: 47. 1953.

In the membrane filter technique of enumerating bacteria, organisms can be concentrated on the filter from aqueous solutions or gaseous substances. The thin filter then is placed on an absorbent pad with added nutrient broth. After 4-8 hrs. incubation in a humid atmosphere colonies can be observed under low power magnification. In 12 hrs. colonies usually can be seen with the unaided eye. The method is particularly adapted to examinations where low numbers of bacteria are involved, and where customary procedures would fail to detect their presence. Figures illustrate the equipment used and comparative data are given for artificially infected water and CIP pipelines.

T. J. Claydon

991. Speciation within the genus Brucella. II. Evaluation of differential dye, biochemical, and serological tests. M. J. Pickett, E. L. Nelson, and J. D. Liberman. J. Bacteriol., **66**: 210-219. 1953.

The separation procedures were evaluated on the basis of the reactions of 232 cultures which were smooth by the acriflavine agglutination test. On the basis of thionin, fuchsin, crystal violet, pyronin, urea and carbamate tests, 214 strains were separated into the usual three species; some other strains required serological examination for adequate identification. With rare exceptions, only *B. melitensis* strains gave positive carbamate tests and only strains of *B. suis* were strongly urea positive. Tests for H_2S did not aid in identification. Use of azure A and safranin O, especially the former, supplemented the traditional four dyes in identifying atypical strains. F. E. Nelson

992. Effect of excess amino acids on growth of certain lactobacilli. A. E. Teeri and D. Josselyn. J. Bacteriol., **66**: 72-73. 1953.

Excesses (20 mg./10 ml.) of valine, serine or histidine inhibited *Lactobacillus arabinosus. L. fermenti* was greatly repressed by excess serine. *L. casei* was inhibited markedly by arginine, histidine and aspartic acid and prevented from growing by glutamic acid. Serine prevented growth of *L. leichmannii,* aspartic and glutamic acids were markedly inhibitory and valine and methionine somewhat inhibitory.

F. E. Nelson

993. Summary of foodborne, waterborne and other disease outbreaks. C. C. Dauer, Pub. Health Service. Pub. Health Reports, **68**, 7: 696-702. 1953.

A total of 163 foodborne and waterborne disease outbreaks, involving 8191 cases, was reported for 1952. Only 6 outbreaks (833 cases) were traced to milk or milk products. Three of these were from milk. *Streptococcus faecalis,* viridans group, was recovered from raw milk produced and served at an institution where the raw milk had been the cause of one outbreak. A large outbreak of *Shigella sonnei* dysentery occurred at a school using milk found to be improperly pasturized. The source of the infection was not determined. In the third instance milk was suspected of being the cause of an outbreak of 100 cases of gastroenteritis in a school. The three outbreaks due to milk products were small. A group of 5 people became ill after eating some cheese; another 7 persons, after drinking egg nog found to contain a salmonella organism; and the third, of 10 people, after eating ice cream contaminated with *Salmonella montevideo.*

Contaminated water was responsible for 14 outbreaks involving 530 persons. The remaining 143 outbreaks affecting 6828 persons were due to foods other than dairy products. Faulty methods of handling, storage, and refrigeration of foods were found to be responsible for many foodborne disease outbreaks. D. D. Deane

994. Thiamine-synthesizing bacteria isolated from cow stomach. T. Koyanagi, T. Hino, and H. Matsui, Univ. Tokyo. Igaku to Seibutsugaku, **11**: 187-190. 1947. (Chem. Abstr., **47**: 9411h. 1953.) B. L. Larson

995. Decomposition of cellulose by microorganisms. H. Siu and E. T. Reese, U. S. Army QMC, Philadelphia, Pa. Botan. Rev., **19**: 377-416. 1953. (A review.) (Chem. Abstr., **47**: 9418d. 1953.) B. L. Larson

997. Action of biotin on propionic acid fermentation. M. Antila, Univ. Helsinki, Fin. Suomen Kemistilehti, 26B, 3: 32-34. 1953. (Chem. Abstr., **47**: 10146g. 1953.) S. Patton

998. Milking ointments containing quaternary ammonium compounds. E. BENK. Seifen-Öle-Fette-Wachse, 79: 289-290. 1953. (Chem. Abstr., 47: 10178h. 1953.) S. Patton

999. The formation of biacetyl in cream, butter, and cheese. J. DOLEZÁLEK, Vysoká skola zem. a les. inz., Prague, Czech. Sborník Ceskoslov. Akad. Zemedelske, 25: 429-440. 1952. (Chem. Abstr., 47: 9516h. 1953.) S. Patton

DAIRY CHEMISTRY

1000. A chromatographic method for the determination of cobalt in feeds. R. P. KING, D. W. BOLIN, W. E. DINUSSON, and M. L. BUCHANAN. J. Animal Sci., 12, 3: 628-634. 1953.

A chromatographic method for the determination of Co in feeds is described. Advantages over previous methods are that Cu and Fe do not interfere with the determination of Co, and larger samples may be ashed to give a greater concentration of Co in the final solution for a colorimetric determination. The Co contents of several feedstuffs are presented.

O. T. Stallcup

1001. Iso-oleic acids in cow and buffalo milk fat. S. S. PHATAK and V. N. PATWARDHAN. Nature, 172, 4375: 456. 1953.

While endeavoring to develop a method for detecting the adulteration of fat from the milk of the cow and buffalo, by paper chromatography of the dibasic acids obtained on oxidation, the authors found spots for suberic, azelaic, sebacic, undecanedioic and dodecanedioic acids. The dibasic acids were obtained in 2 ways and both methods gave identical chromatograms. The conclusion is drawn that in addition to oleic acid, the fat from the milk of the cow and buffalo contains other octadecanoic acids, with double bonds at the 8, 10, 11 and 12 position. The authors speculate that the positional isomers of oleic acid are the sources of the dibasic acids identified in this investigation.

R. Whitaker

1002. Colorimetric determination of reconstructed milk in normal milk. A. REINART and R. W. BROWN. Can. Dairy and Ice Cream J., 32, 6: 30-31. 1953.

For the quantitative detection of reconstituted milk in normal milk, a colorimetric method has been developed by modifying Evenson's color test for remade milk and cream. This method is based on the fact that the curd of the milk sample, when treated with acetone and ethyl-ether during washings, dissolves in sodium-hydroxide solution and gives a transparent solution which after filtering and heating becomes clear. The depth of the yellow color of the sample, which develops when the sample contains reconstituted milk, is measured and the amount of reconstituted milk added determined by means of an electrophotometer or color standards. H. Pyenson

1003. Microbiological assay of vitamin B complex including folic acid in buffalo milk. T. J. BOMAN, Public Health Lab., Poona. Indian J. Dairy Sci., 6, 2: 41-45. 1953.

Buffalo milk contained: thiamine 0.50, riboflavin 1.02, nicotinic acid 2.60, biotin 0.13, pantothenic acid 1.50, pyridoxine 3.25, para-aminobenzoic acid 26.75, and folic acid 5.50 γ / ml.

W. C. van der Zant

1004. Electrophoretic studies of milk. III. Researches on cow milk at the end of lactation. G. V. HEYNDRICKX and A. DE VLEESCHAUWER, State Agr. Univ., Ghent, Belg. Bull. soc. chim. biol., 34: 1016-1025. 1952. (Chem. Abstr., 47: 8871b. 1953.) S. Patton

1005. Mucoproteins of human milk and cow's milk. L. FERRANTE, Univ. Ferrara, Italy. Acta pediat. latina (Parma), 6: 80-87. 1953. (Chem. Abstr., 47: 8880a. 1953.) S. Patton

1006. Determination of citric acid in milk. R. COPPENS, Inst. agron. état, Gembloux, Belg. Bull. inst. agron. et stas recherches Gembloux, 17: 16-25. 1948-49. (Chem. Abstr. 47: 8921c. 1953.) S. Patton

1007. Volumetric determination of proteins in cow milk. A. BOSTICCO, Univ. Turin, Italy. Osped. maggiore Novara, 29: 187-191. 1952. (Chem. Abstr., 47: 8921h. 1953.) S. Patton

1008. Lactose as an index of diluting milk. G. D'ALÒ, Uff. Igiene, Pesaro, Italy. Igiene moderna, 45: 285-302. 1952. (Chem. Abstr., 47: 8921i. 1953.) S. Patton

1009. Composition of milk of Indian animals. III. Freezing point and lactose and chloride content of milk samples from different farms in India. N. N. DASTUR, C. S. DHARMARAJAN, and R. V. RAO, Indian Dairy Research Inst., Bangalore. Indian J. Vet. Sci., 22:123-133. 1953. (Chem. Abstr., 47: 9516e. 1953.) S. Patton

1010. The determination of milk fat and coconut oil in chocolate products. O. SCHETTY and M. VAUCHER. Rev. intern. chocolat., 8: 101-106. 1953; cf. Hadorn and Jugkunz, ibid., 7: 358-360. 1952. (Chem. Abstr., 47: 9520d. 1953. S. Patton

1011. Filter papers and the phosphatase test. G. T. COOK and K. E. A. HUGHES, Pub. Health Lab., Portsmouth, Engl. Dairy Ind., 18: 43-45. 1953. (Chem. Abstr., 47: 10143e. 1953.) S. Patton

1012. Calcium salts and the rennet coagulation of milk. G. T. PYNE, Univ. Coll., Cork, Ire.

Chemistry & Industry, 302-303. 1953. (Chem. Abstr., **47**: 10143f. 1953.) S. Patton

1013. Electrophoretic studies on milk proteins. I. A. LEMBKE, W. KAUFMANN, and H. GANTZ, Bakt. Inst. Milch., Kiel, Ger. Kiel. Milchwirt. Forschungsber., 4: 571-578. 1952. (Chem. Abstr., **47**: 10143h. 1953.) S. Patton

1014. Flame photometry in the determination of potassium, sodium, and calcium in milk. G. SCHWARZ and B. DRAUSZ, Inst. landw. Technol., Stuttgart Hohenheim, Ger. Kiel. Milchwirt. Forschungsber., 4: 579-593. 1952. (Chem. Abstr., **47**: 10144i. 1953.) S. Patton

1015. Indicator substances for the detection of hydrogenated oil when used as adulterant of clarified butterfat. II. R. S. DEWAN, A. R. S. KARTHA, and K. C. GULATI, Indian Agr. Research Inst., New Delhi. J. Sci. Ind. Research (India), 12B: 178-179. 1953. (Chem. Abstr., **47**: 10249g. 1953.) S. Patton

1016. Report of the peroxidase reaction. CCLXIV. *1* and *2*. Isolation and identification of methylglyoxal (pyruvaldehyde or pyruvic aldehyde) from human milk. 3. Identification of acetaldehyde and methylglyoxal from human milk by paper partition chromatography. H. WAKO, Tohoku Univ., Dendai, Japan. Tohoku J. Exptl. Med., 57: 181-190; 191-197. 1953. (Chem. Abstr., **47**: 10078g. 1953.) B. L. Larson

DAIRY ENGINEERING

1017. Pyramid-type paper bottles made, filled link-fashion. Food Eng. Staff, Food Eng., **25**, 9: 48-49. 1953.

The Tetra-Pak is a paper container in tetrahedron form being used in a number of Swedish dairies. The forming-filling machine is made by AB Tetra Pak, Lund, Sweden, and is installed on a lease basis. Paper is fed from a roll into the top of the machine and is formed into a tube as it moves downward. The tube is sealed longitudinally and is filled with milk. Heat sealing jaws press the tube together at intervals, sealing it into uniform packages. The linked packages go to a cutting unit where they are separated and cartoned in six-sided fiber cases. The process results in considerable saving in paper, labor, storage, and floor space. T. J. Claydon

1018. What's new in bulk transportation? T. A. BURRESS, Heil Co. Milwaukee, Wis. Milk Plant Monthly, **42**, 9: 30-31. 1953.

Major improvements in tandem design rank high on the list of profit-making developments currently being applied to bulk milk handling. One of these is the self-steering spread tandem. It uses widely spaced axles to carry greater legal payloads than are permissible with the conventional 48-in. tandem. The increased allowance in some states may be 4,000 lbs. There are now 2 types of articulating spread tandems in use. The most recent has a spacing of 9 ft. 2 in. between front and rear axles, and the rear axle is free to steer as much as 10° in either direction. This type of spread tandem permits a net allowable load increase of some 3,500 lbs. or a bonus pay load of 400 gal. of milk every trip. The other articulating spread tandem is one on which the front axle, rather than the rear, is free to steer. The axles are spaced 9 ft. 1½ in. apart, and permit a payload gain of about 250 gal. C. J. Babcock

1019. Principles underlying the design of a balanced refrigerator system. L. BUEHLER, JR., Creamery Pkg. Mfg. Co., Chicago, Ill. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 63-67. 1953.

The basic principles of refrigeration are discussed. It is desirable to strike a balance between the amount of condenser water and the quantity of electric power, depending on the unit cost of water and power. In an oversize ammonia compressor, for a particular cooling job the operating pressure and temperature will be pulled down below the level desirable for best economy. J. Tobias

1020. Problems encountered in the care and lubrication of a refrigeration machine. E. D. ROBERTS, Socony-Vacuum Oil Co., Detroit, Mich. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 68-73. 1953.

In order to get best results from any system attention should be given to the following considerations: (1) An oil having the necessary characteristics to suit it well for the type of refrigerant used should be selected, (2) the system should be kept free of rust preventives, pipe and gasket compounds, dirt, scale rust and water, (3) scale traps, oil drains and traps and oil separators should be kept drained, clean and in proper working order, (4) clean and dry transfer containers should be used for adding oil, (5) a record should be kept of critical temperatures and pressures, (6) oil feed should be maintained at adequate minimum rate. A record of oil consumption is desirable, (7) oil batches in compressor and auxiliary units should be changed often enough to secure economical life of charge, (8) all heat transfer surfaces should be maintained in a clean state, and (9) good care should be taken of compressor drive units, pumps, circulating fans and other auxiliary equipment. J. Tobias

1021. Elimination of frost difficulties in a low temperature refrigeration system. K. A. NESBITT, Westerlin & Campbell Co. Detroit, Mich. Proc. First Ann. Dairy Eng. Conference, Mich State Coll., East lansing. p. 76-80. 1953.

Merits of a number of defrosting procedures are discussed. Included are hot gas, brine spray, water spray, warm air and electric procedures.
J. Tobias

1022. How truthful is your temperature control equipment? J. E. BARBER, Taylor Instrument Co., Rochester, N. Y. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 92-96. 1953.

Accuracy of thermometers may be measured under both static and dynamic conditions. Under static conditions accuracy is frequently expressed as the maximum inaccuracy in % of full scale range. Dynamic accuracy may be expressed as the time by which the recorder lags the true temperature or the 63% response time. Examples are cited on the accuracy of dairy thermometers. J. Tobias

1023. Where are our BTU's Going? L. C. THOMSEN, Dept. Dairy & Food Ind., Univ. of Wis., Madison. Proc. First Ann. Dairy Eng. Conference. Mich. State Coll., East Lansing. p. 85-91. 1953.

Some suggestions are given to effect economies in fuel. Systems discussed included long hold pasteurization, HTST pasteurization and vacuum evaporation. J. Tobias

1024. Homogenizer. B. FELDMANN. U. S. Patent 2,652,234. 2 claims. Sept. 15, 1953. Official Gaz. U. S. Pat. Office, 674, 3: 733. 1953.

A homogenizer valve assembly, having as an important feature a shaft extending down from the tapered valve through the inlet tube. The shaft is grooved with a spiral channel.
R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

1025. Economic aspects of milk industry. L. C. SPENCER, Cornell Univ., Ithaca, N.Y. Sou. Dairy Prod. J., **54**, 3: 134-136. 1953.

It is the opinion of the author that the trade barrier problems related to milk and cream have diminished in recent years. High transportation costs are generally the barriers which curtail long shipments of milk. Artificial barriers do affect the marketing of cream in some instances. Regulations in New York State which limit the sources of fluid cream result in higher prices and tend to decrease consumption, but the effect of this factor upon surpluses of dairy products is sometimes exaggerated. Formulas for determining minimum prices which Boston and New York dealers must pay for milk include supply-demand adjustment factors and have resulted in considerable reduction in the prices which dealers pay for fluid milk.
F. W. Bennett

1026. Bulk tank pick-up system. K. SANDERS, The Heil Co., Milwaukee, Wis. Milk Dealer, **42**, 11: 38-39. 1953.

A survey of the Nation's dairy processors shows that: (1) the bulk tank pick-up system presently accounts for probably less than 5% of all milk pick-up in the United States, with the exception of Calif. where the majority of the milk is handled this way; (2) the farm pick-up program has been growing rapidly since the end of World War II; (3) although the great majority of the dairies do not at present operate tank pick-up routes, a very significant proportion (40-45%) are seriously considering it; (4) the savings which would accrue to a dairy with 100% tank pick-up is by far the most important reason this system is being considered; (5) the small size of milk producers seems to be the primary reason for resistance to adoption, and (6) the dairy processor will account for almost ½ of the sales of tank truck equipment and contract haulers will buy their own tank equipment about 50% of the time.
C. J. Babcock

1027. What happens to your milk? H. V. ATHERTON, F. J. DOAN, and G. H. WATROUS, JR., Pa. Agr. Expt. Sta., State College. Milk Plant Monthly, **42**, 9: 15-17. 1953.

A limited survey of retail milk outlets in 2 small communities of central Pa. revealed that most of the self-service cabinets employed by stores and supermarkets are unsatisfactory in that milk is not held at sufficiently low temperatures to prevent deterioration. Small outlets (1 to 3 cases of milk) are holding milk colder than the large elaborately equipped stores. The small outlets generally employ completely enclosed refrigerated boxes and soft drink or ice cream cabinets. Most store operators have little idea of the efficiency of their milk cabinets and only a vague knowledge of how bottled milk should be handled. In view of the increasing importance of the store in milk distribution and the relation of temperature to keeping quality of bottled milk, it appears that milk dealers selling through stores might profitably examine the facilities and methods used in the holding and handling of their milk. The consumer who gets milk of poor keeping quality is more likely to blame the dairy whose name is on the container than the store which sells the product.
C. J. Babcock

1028. Save time in your dairy plant for profit. C. W. HALL, Mich. State Coll., East Lansing. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 1-5. 1953.

Time and motion studies can improve the operation in practically every part of the dairy plant. Savings are accomplished by combining, eliminating, simplifying, and changing the sequence of operations. Specific examples are given. J. Tobias

1029. Benefits of time and motion studies in ice cream plant operations. A. H. BAYER and

F. M. SKELTON, General Ice Cream Corp., Schenectady, N. Y. Proc. First Ann. Dairy Eug. Conference, Mich. State Coll., East Lansing. p. 6-11. 1953.

A program referred to as "Methods Improvement or Work Simplification" at the General Ice Cream Corporation is discussed. The success of the program is dependent on maintaining a questioning attitude on all phases of the operation by the men doing the job. Workers are given an opportunity to express their ideas during problem-solving conferences. Improvements are also made through careful study of flow process charts. When a better method is developed at one plant, movies of it are taken before and after the improvement and these are shown in other plants. Examples of specific improvements are given. J. Tobias

1030. Air conditioning in a dairy plant. A. PETRONIS, Carrier Corp., Detroit, Mich. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 52-54. 1953.

The bottle and carton filling room in the milk plant could benefit by controlled atmospheric conditions. Maintaining a temperature of 75-80° F. and 40-50% relative humidity will cause a saving in scrap and returns.

Accepted conditions for a cow barn are 80° F. and 60% relative humidity. A cow gives off approximately 3500 BTU per hour.

In the cheese industry the control of temperature and humidity is of utmost importance. Cottage cheese vats should be maintained at approximately 70° F. Loss in weight due to evaporation will occur in hard cheese during curing when humidity is not properly controlled.

Mechanical ventilation and air filtering are suitable for the pasteurizing area, case rooms or raw milk storage. J. Tobias

1031. Looking ahead in the dairy industry. H. F. JUDKINS, National Dairy Prod. Co., Inc., New York, N. Y. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 55-62. 1953.

New products, processes, and equipment which have contributed to the growth of the dairy industry are discussed. Developments which the industry will need in the future are enumerated. J. Tobias

1032. Savings effected through efficient use of condenser water. D. K. WEBER, Heatherwood Farms Co., Lansing, Mich. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 74. 1953.

To combat a shortage and eventual increased cost of water, a cooling tower was erected to save water which will pay for the additional equipment in 5 yrs. time. J. Tobias

1033. Milk losses in dairy plants. J. W. RUGABER. Can. Dairy and Ice Cream J., **32**, 6: 40-48. 1953.

Leakage, overflow, spillage, wilful waste, residual waste, and carry over are important points to consider in the prevention of dairy plant waste. H. Pyenson

1034. How much is my ice cream business worth? P. B. BECK, Mgr., Dept. of Statistics and Accounting, International Assoc. of Ice Cream Manufacturers, Washington, D. C. Ice Cream Rev., **37**, 1: 40. 1953.

There is no formula for establishing the value of a business. Its value in terms of the buyer will be influenced by the extent to which the existing facilities can be utilized if merged with those of the purchaser. If the building and equipment can be utilized in their present location their value to the purchaser will depend upon such factors as: location of the property from the standpoint of procurement of ingredients and supplies, accessibility to employees, proximity to the market, condition and arrangement of the equipment, possibility for increasing capacity of the plant, and condition and arrangement of hardening rooms.

In addition to the building and processing equipment, delivery equipment and cabinets should be given careful consideration as to age and condition.

Customers and company personnel are valued largely by the earning power of the company. The value of the customers will be determined by the average annual gallonage, the location of the accounts, and the soundness of the price structure. The value of the company personnel is something the purchaser alone will determine.

The average operating profit is considered the best single guide for determining the good will value of the business.

Careful consideration of these factors will assist in arriving at a value mutually acceptable to the buyer and seller. W. J. Caulfield

1035. Operational costs in a dairy plant—*1952*. C. W. HALL, Mich. State Coll., East Lansing. Mich. Agr. Expt. Sta. Quart. Bull., **36**: 107-129. 1953.

Several graphs are presented showing daily and yearly costs of operating various types of plant equipment. Size of operation also is considered. R. W. Hunt

FEEDS AND FEEDING

1036. Comparison of two isotope methods for determination of endogenous fecal calcium. C. L. COMAR, R. A. MONROE, W. J. VISEK, and S. L. Hansard. J. Nutrition, **50**, 4: 459-467. 1953.

Comparative values are given for endogenous fecal Ca in cattle by an isotope dilution method and a comparative balance method. These procedures, though different in principle, make use of labeled Ca. The isotope dilution method has the advantage of being independent of the availability of the dietary Ca and is devoid of

the uncertainties of balance determinations. Schematic drawings are presented to illustrate the principle involved in each of these methods.
O. T. Stallcup

1037. The role of plant pigments in digestion trial studies. H. M. Irvin, H. G. Wiseman, J. C. Shaw, and L. A. Moore. J. Animal Sci., **12**, 3: 541-551. 1953.

The authors present a review of literature to indicate that carotenoids and xanthophylls are too unstable and too digestible for markers in digestion trial studies. Chlorophyll, although definitely changed, may be retained in the digestive tract in sufficient amounts to be used as a marker. Data are presented which indicate that the composite pigment picture in hay and feces contains a similarity which supports the basic theory of the chromogen marker technique. The authors concluded that no one pigment can be used as a digestion trial marker but the individual pigments, although varying greatly between hay and feces, may produce composite absorption curves comparable with one another.
O. T. Stallcup

1038. Studies on vitamin A in milk. VI. The influence of continued intake of shark-liver oil on the vitamin A content of milk and butterfat. K. M. Narayanan, C. P. Anantakrishman, and K. C. Sen, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **6**, 2: 67-74. 1953.

Shark liver oil *(I)* was fed to cows and buffaloes at the rate of 100,000-200,000 I.U./head/day in 2 experiments for a period of 7 and 22 wk. *I* did not show any effect on either milk yield or fat %. On a grass ration with a daily carotene intake ranging from 1241-1385 mg./head *I* produced no beneficial effect on the vitamin A content of milkfat. When the daily carotene intake was lowered to 300 mg./head *I* showed a temporary increase in the vitamin A content of milkfat. *I* caused a slight decrease in the carotene content of the milkfat of cows. On a dry regime (ragi straw) 100,000-200,000 I.U. of vitamin A/day increased the vitamin A content appreciably and continued supplementation maintained this high level of vitamin A in both cows and buffaloes. On a dry regime the recovery of vitamin A in the milkfat of cow and buffalo amounted to 4.5 and 6.7% resp.
W. C. van der Zant

1039. Vitamin supplements for dairy calves. S. R. Skaggs. N. M. Agr. Expt. Sta. Bull., 376. 1953.

Twenty unselected Holstein calves were divided into 4 groups: (1) control, (2) 25,000 IU vitamin A daily, (3) 25,000 IU vitamin A plus 50 mg. niacin, (4) 30,000 IU vitamin A and 25,000 IU vitamin D plus 50 mg. niacin. All calves were fed colostrum milk for 3 d., whole milk until 30 d. of age when all milk feeding was discontinued. Supplementing with vitamin A alone resulted in lower feed consumption and reduced growth. Vitamin A plus niacin increased feed consumption but gains were lower than when only vitamin A was used. Those calves receiving vitamin A and niacin plus vitamin D were highest in feed consumption and lowest in growth rate. All calves reached normal weight by the end of 1 yr., but those receiving niacin required more feed. No positive benefits were found from feeding any of the vitamins in this experiment.
R. W. Hunt

1040. Effects of feeding antibiotics to dairy calves. I. Aureomycin and bacitracin feed supplements. S. A. Ellsworth, C. F. Huffman, C. K. Smith, and N. D. Ralston, Mich. State Coll., East Lansing. Mich. Agr. Expt. Sta. Quart. Bull., **36**: 60-65. 1953.

Twenty-one dairy calves were fed 1 mg. of aureomycin (Aurofac) or bacitracin (Bacifirm) in the form of feed supplements for each lb. of weight from 3-100 d. of age. The group fed Aurofac, gained 23% more than did the controls, while the Bacifirm group gained 14%. No distinct differences were observed between the supplemented and non-supplemented groups in regards to coliform and streptococci count of the feces.
R. W. Hunt

1041. Blood levels and retention of calcium, magnesium, and potassium in lambs fed alfalfa hay containing narrow and wide potassium to calcium ratios. H. D. Eaton, J. E. Avampato, G. O. Curme, III, and L. D. Matterson, Univ. of Conn., Storrs. Storrs Agr. Expt. Sta. Bull., 295. 1953.

The results of this work were obtained from two different experiments. The first experiment involved the use of 3 groups of lambs, each containing 4 animals. Each group was assigned at random to one of 4 rations as follows: (1) normal alfalfa, (2) normal alfalfa plus sufficient KCl to make the K equivalent to 4%, (3) high-K alfalfa, and (4) high-K alfalfa plus sufficient KCl to make the K content to 4%. Both whole blood and serum K showed wide variability between lambs in any one group. Serum Ca was higher ($P < 0.10$) in animals fed normal than those fed high-K hay. The addition of KCl to normal hay resulted in significant decreases ($P < 0.05$) in serum Ca, while its addition to high-K hay resulted in no appreciable effect. Ca retained by lambs fed normal hay was greater ($P < 0.10$) than that retained by the lambs fed high-K hay. The addition of KCl to either hay resulted in slight, but not significant, decreases in retention of Ca. K retained was greater in those lambs fed high-K ($P < 0.10$), and the addition of KCl to either hay resulted in marked increase ($P < 0.01$) in retention of this element. Histological examination of tissues from the heart, liver,

spleen, pancreas, kidney, parotid, pituitary, adrenal, thyroid, pineal, and reproductive tract taken upon slaughter at the 12th experimental week, showed no consistent changes due to types of hay fed or the addition of KCl.

The second experiment consisted of feeding (1) normal hay, (2) high-K (hay which was grown on plots which had received high KCl treatments), and (3) alfalfa hay plus KCl to make the K content equal to that of high-K. The average hematocrit value, representing packed red cell volume, was increased on the addition of KCl to the normal alfalfa. There were no marked differences upon feeding either of the hays alone. Both whole blood and serum K reflected the higher K intake of the lambs when fed the high-K hay or the KCl fortified normal hay. Similarly, serum Ca and Mg were greater in the lambs during the feeding of normal alfalfa than high-K. Retention of Ca and Mg was not significantly different for either type of hay fed alone. However, KCl fortified normal hay resulted in significant decreases in the Ca retained and also in a decrease of Mg, but the latter was not significant. K retention was found to reflect K intake. R. W. Hunt

1042. Antibiotics for dairy calves. C. G. M. EDGERLY, Dept. of Animal Ind., Me. Agr. Expt. Sta., Orono. Presented at meeting of E. Div. A.D.S.A. Sept. 20, 1953.

The effects of aureomycin, terramycin and a mixture of equal parts aureomycin, terramycin, bacitracin and procaine penicillin on the growth and health of Holstein, Jersey and Guernsey calves from birth through 12 wks. of age is currently being determined. Calves received antibiotics at rate of 1 mg. per lb. of body weight daily for 12 wks. beginning at 1 d. of age. The antibiotic was mixed with the milk at the evening feeding. Weekly adjustments for weight increases were made. Calves were kept in individual pens and fed ration of 1 lb. whole milk per 10 lbs. body weight to maximum of 10 lbs. daily for Holsteins, and 1 lb. of whole milk per 8 lbs. of body weight to maximum of 8 lbs. daily for Guernseys and Jerseys; grain, free choice to maximum of 4 lbs. daily for Holsteins and 3 lbs. daily for Guernseys and Jerseys; hay, free choice; water was constantly available. Preliminary results with a total of 19 calves show ave. daily gains for control group—1.07 lbs., for aureomycin supplemented group—1.27 lbs., for terramycin supplemented group—1.23 lbs., for antibiotic mixture supplemented group—1.36 lbs. Gains per lb. of TDN were: controls—0.45 lbs., aureomycin group—0.51 lbs., terramycin group—0.47 lbs., antibiotic mixture group—0.51 lbs. Supplemented groups consumed approximately 30% more water.

No serious scours occurred in any group, but all controls had mild scours while only 2 calves receiving antibiotics had mild scours. Calves scouring or suffering from respiratory disorders when separated from their dams made rapid recovery when fed antibiotics. When the calves no longer received the antibiotics normal gains were continued for about 1 wk. followed by a 2-3 wk. adjustment period where there was some loss of weight and a tendency to be unduly laxative, particularly those calves receiving aureomycin and terramycin. After 4 wks. these calves were back to normal and making equal gains with the controls. The group that received the antibiotic mixture was not upset as much in this adjustment period and it made better gains after removal from the antibiotic feed.

Author's Abstract

1043. Het vetgehalte in het voedermiddel en het vetgehalte der melk (Fat in feeds and the fat content of milk). J. H. J. VAN LAARHOVEN. Officieel Orgaan van de Koninklyke Nederlandse Zuivelbond, **45**: 564-565. 1953.

A supplementation of the ration with 125 g. stearic acid increased the fat content of the milk approx. 0.2%; higher supplements did not show additional increases. The economical aspects of this type of supplementation is discussed. W. C. van der Zant

1044. Ammoniated feeds. Ammoniated industrial by-products in dairy heifer rations. N. D. MAGRUDER, C. B. KNODT, and P. S. WILLIAMS, Pa. Agr. Expt. Sta., State College. Ag. and Food Chem., **1**, 15: 944-946. 1953.

The supplementation of an ammoniated industrial by-product, hemicellulose extract (wood sugar derivative), at the 10% level in a grain ration for growing Holstein heifers was comparable in body weight gains and digestibility coefficients for crude protein (N X 6.25) to a similar grain ration supplemented with soybean oil meal. Ammoniated cane molasses at levels of 50% of the grain ration was not toxic when fed to a yearling dairy heifer. Many industrial plants in the United States have by-products that have been dumped into streams as wastes, causing pollution. If these by-products, upon ammoniation, can be used as economical nitrogen and energy extenders for ruminants, industrial plants, feeders of cattle, and consumers may benefit. S. Patton

1045. Detection of estrogenic substances in alfalfa and clover hays fed to fattening lambs. E. CHENG, C. D. STORY, L. C. PAYNE, L. YODER, and W. BURROUGHS. J. Animal Sci., **12**, 3: 507-514. 1953.

Estrogenic activity in alfalfa and clover hays was assayed against standard doses of stilbesterol by noting the stimulation in uterine weights of immature mice. The potency of the clover and alfalfa hays varied from 0.8 to 2.7 mcgs. of stilbesterol activity/lb. of hay tested. Blue grass, brome grass, ladino clover, immature wheat planto, corn and wheat grains, and soybean oil meal failed to show estrogenic

activity by the above assay. The possible significance in feeding practice of these levels of estrogens in hays is discussed.
O. T. Stallcup

1046. Cattle rations. L. A. Henke. Hawaii Agr. Expt. Sta. Biennial Rept., p. 29. 1950-52.
Studies in the economy of raising calves on milk substitutes during their first 6 mo. showed slower gains in weight (1.13 vs. 1.39 lbs. per d.) but marked economy in dairy feed cost (29¢ vs. 30¢). At 30 mo. of age, 38 milk-substitute-fed calves weighed 99.9% as much as the milk-fed. Milk production records are now being determined as these animals come into production. Ninety-seven animals are included in the study.
R. W. Hunt

1047. Chemical composition of hay in barn-drying. R. B. Davis, Jr., V. H. Baker, P. M. Reaves, J. F. Eheart, and W. N. Linkhous. Agr. Eng., **32**: 218-220. 1951. (Chem. Abstr., **47**: 10149e. 1953.) S. Patton

1048. The chemical composition and nutritive value of Welsh hay in comparison with other British hays. II. W. M. Ashton and D. E. Morgan, Univ. Coll. Wales, Aberystwyth. J. Brit. Grassland Soc., **8**: 131-147. 1953. (Chem. Abstr., **47**: 10149d. 1953.)
S. Patton

1049. The nutritive value of some Queensland fodders. J. M. Harvey, Dept. Agr. and Stock, Brisbane, Australia. Queensland J. Agr. Sci., **9**: 169-184. 1952. (Chem. Abstr., **47**: 10149f. 1953.) S. Patton

1050. The nutritive value of grass protein. I. The isolation of proteins from the leaves of red clover and orchard grass. M. Kandatsu and H. Nishi, Univ. Tokyo. J. Agr. Chem. Soc. Japan. **25**: 562-566. 1951-52. (Chem. Abstr., **47**: 8786i. 1953.) S. Patton

1051. The digestion of the pulp of dried sugar beets by cattle. A. M. Leroy, G. Lery, and S. Zelter, Natl. agron. inst., Paris. Ann. inst. natl. recherche agron., Sér. D. Ann. zootech., **1**, 1: 23-28. 1952. (Chem. Abstr., **47**: 8863i. 1953.) S. Patton.

1052. Estimation of relative productive values of cattle food. A. K. B. Cazi, Univ. Bombay. J. Univ. Bombay, Sect. B, 20, Pt. 5, **31**: 24-33. 1952. (Chem Abstr., **47**: 8927b. 1953.)
S. Patton

1053. Chemical composition and digestibility of grassland crops. T. Homb, Roy. Agr. Coll., Norway, Vollebekk. Acta Agr. Scand., **3**: 1-32. 1953. (Chem. Abstr., **47**: 8927e. 1953.)
S. Patton

1054. Digestibility of beechwood holocellulose by ruminants. W. Klauditz and M. Becker, Inst. Holzforschung, Braunschweig, Ger. Holzforschung, **6**: 103-104. 1952. Chem. Abstr., **47**: 8927e. 1953.) S. Patton

1055. Ensilage of sugar-beet pulp: A bacteriological and biochemical study. I. Laboratory research. A. Barret, I. Glaser, and J. L. Perroton, Plant Technol. Sta., Versailles, Fr. Ann. inst. natl. recherche agron., Sér. E. Ann. technol. agr., **1**, 1: 133-159. 1952. (Chem. Abstr., **47**: 8927f. 1953.) S. Patton.

GENETICS AND BREEDING

1056. Viable genetic hypotrichosis in Guernsey cattle. F. B. Hutt and L. Z. Saunders, N. Y. State Coll. of Agr. and N. Y. State Vet. Coll., respectively, Ithaca. J. of Heredity, **44**, 3: 97-103. 1953.
The cases of 2 closely related Guernsey calves born with extreme hair deficiencies were concluded to be caused by a simple autosmal recessive gene that is different from the 3 genes causing *lethal hairlessness, semi-hairlessness* and *hypotrichosis with adontia.* The principal characteristics differentiating viable hypotrichosis from the other 3 hypotrichosis traits are viability and the normal teeth and hoof development. The abnormal follicle lacked differentiation of the lining epithelial cells into Henle's layer, Huxley's layer and the cuticle. The tubular (sweat) glands were cystic. The sebaceous glands were normal. All types of hypotrichosis are distinctly different from *epitheliogenis imperfecta.* A detailed review of literature is included. L. O. Gilmore

1057. Predicting the transmitting ability of dairy sires. H. W. Hall and H. C. Dickey, Univ. of Me., Orono. Presented at meeting of E. Div. A.D.S.A. Sept. 20, 1953.
Five methods of predicting the transmitting ability of dairy sires were examined statistically using 531 pedigrees with complete information. All of the methods gave highly significant correlation coefficients when the actual and the predicted production of the dairy sires' daughters were compared. Three of the 5 methods were superior in that their correlation coefficients were much greater, and their average deviations and standard errors of estimate from actual were smaller.
The 3 best methods in this study were (1) Graves' method of weighting the ave. equal-parent sire index of the sire, maternal grandsire, and maternal great grandsire; (2) Rices' regressed method using 50% of the sire's regressed index, 25% of the maternal grandsire's regressed index, 10% of the maternal grandsire's regressed index, 10% of the dam's record, and 5% of the maternal grandam's record; and (3) Dickey's regressed method using 50% of the sire's regressed index and 50% of the

dam's regressed record of production. The 3 above methods obtained pedigree values which were then averaged with the mates' records to predict the daughters' records.

Author's Abstract

HERD MANAGEMENT

1058. An economic analysis of large dairy farms, New York, 1949-50. A. J. ASHE. Cornell Univ. Agr. Expt. Sta., Ithaca, N. Y. Bull. 887. 1953.

One hundred and fifty-one large dairy farms were surveyed. The average age of the operators was 47; 1 out of 5 had attended or graduated from college. One out of 4 was in partnership. Herds ranged from 41 to 246 cows, with a mean production of 8,505 lbs. Average farm acreage was 512, of which 173 was in crops. Capital investment centered around $72,518; receipts amounted to $36,486, with expenses amounting to $29,788. Average labor income was $3,072, but 1 out of 4 farms had a minus labor income. Production costs of 100 lbs. of 3.7% fat milk was $4.10, which was 3¢ more than the price received. Size of business was one of the most important factors affecting success. Efficient use of labor had a decided influence on costs and return. Farmers located in valleys made higher labor incomes than did those on hills, because of higher rates of crop and livestock production. R. W. Hunt

1059. How to reduce labor, steps and costs in dairying. H. M. YOUNG, JR., and G. R. BYERS. Coll. of Agr., Univ. of Ky., Lexington. Circular 505. 1953.

Reasons for dairy expansion, machine milking vs. hand milking, work routines, building and equipment, suggested methods for improvement, comparison of building cost, bedding, and advantages and disadvantages of arrangements, are the topics covered in this circular.

R. W. Hunt

1060. Packaged silage—Will plastics replace silos? ANON. State Univ. of N. J., Rutgers. N. J. Agr., **35**: 4-5. 1953.

Two large scale silage projects are using plastic covers. One experiment consists of two 100 ton stacks, packed by using a small tractor, then covered with a plastic film with the edges of the plastic cover buried in a trench dug around the periphery. The second is a trench silo filled with 350-400 tons of silage and covered with vinyl plastic. In both cases, the silage is reported to be keeping well. A substantial saving in cost of storage is expected in both instances. Consideration is being given to filling plastic bags in the field. A 4′ × 8′ bag will hold a ton of silage. R. W. Hunt

1061. Measuring device for farm bulk tanks. W. M. STANHOUSE, S. S. HUNTER, Inc., Syosset, N. Y. Milk Products J., **44**, 10: 36. 1953.

A brief description of a liquid level indicator developed specifically for the farm bulk milk storage tank and for mixing tanks is presented. It operates electrically and is designed for improved sanitation, accuracy and low cost.

The essential parts of this device are a 36 in. clear plastic probe containing a calibrated scale, a stainless steel base and a neoprene "umbrella" which forms a sanitary closure over the tank opening. J. J. Janzen

1062. Conduit connection for milking machines. E. B. THOWPSON (assignor to Globe Hoist Co.) . U. S. Patent 2,653,567. 3 claims. Sept. 29, 1953. Official Gaz. U. S. Pat. Office, **674**, 5: 1222. 1953.

A small portable self-contained milking machine, complete with a power unit and a milk receiving vessel and cover. The cover and connecting tubes are so constructed and arranged as to properly attach to the vessel only when the power unit is supported and in the operative position. R. Whitaker

1063. This calf will be a cow—Give her the care she needs. J. E. COUEY, L. J. BOYD, and J. W. FOSTER. Coll. of Agr., Univ. of Ky., Lexington. Circular 507. 1953.

This circular recommends methods for the housing, care, and feeding of calves and their dams. The outlined recommendations are illustrated by numerous photographs.

R. W. Hunt

1064. Mechanized feeding mechanism for animals. W. R. PETERSON and A. C. RADTKE (assignors to International Harvester Co.). U. S. Patent 2,654,344. 10 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 127. 1953.

Baled feed is shredded by a mechanized device and discharged onto an endless conveyor belt traveling along in front of a line of cows in stalls in a barn. R. Whitaker

1065. Dairy feeder. C. E. IDDINGS. U. S. Patent 2,652,026. 1 claim. Sept. 15, 1953. Official Gaz. U. S. Pat. Office, **674**, 3: 673. 1953.

A device for moving dry feed for cattle from a bin to a trough by means of a screw conveyor.

R. Whitaker

1066. Nipple-equipped feeder pail. J. M. COYNER. U. S. Patent 2,652,027. 6 claims. Sept. 15, 1953. Official Gaz. U. S. Pat. Office, **674**, 3: 674. 1953.

A pail with an [illegible] on the side near the bottom, equipped [illegible] an elbow shaped attachment, for complete [illegible]nage of the pail through a nipple for feeding [illegible]alves. R. Whitaker

1067. Can submerging device for milk coolers. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,654,500. 4 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 172. 1953.

Empty milk cans are held in a submerged condition in a cooling tank by an arrangement of bars which hook over the handles of the cans. The handles must be of the fixed rather than the hinged type. One or more cans may be inserted in or removed from the cabinet without disturbing the other cans. R. Whitaker

1068. Calf puller. W. L. Matteson, Jr. U. S. Patent 2,654,368. 1 claim. Oct. 6, 1953. Official Gaz. U. S. Pat. Office **675**, 1: 135. 1953.

A mechanical device for attaching to the rump of a cow for assisting in the delivery of a calf. R. Whitaker

1069. Milk can hoist with friction brake. J. J. Voda (assignor to International Harvester Co.). U. S. Patent 2,654,570. 6 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 193. 1953.

A hand operated hoist for lifting milk cans in and out of a cooling tank. R. Whitaker

1070. Pulsator for milking machines. F. J. J. J. Henrard (assignor to Eeremeuses Melotte). U. S. Patent 2,655,168. 14 claims. Oct. 13, 1953. Official Gaz. U. S. Pat. Office, **675**, 2: 405. 1953.

Details are given for the construction of a device for creating pulsations in the vacuum supply for a milking machine.

R. Whitaker

1071. Animal restraining device. C. H. Basler and W. L. Basler. U. S. Patent 2,655,900. 4 claims. Oct. 20, 1953. Official Gaz. U. S. Pat. Office, **675**, 3: 663. 1953.

An apparatus, clamped on the back of a cow, for preventing the animal from kicking.

R. Whitaker

1072. Thermostatically controlled milk cooler. W. Hurley. U. S. Patent 2,656,154. 1 claim. Oct. 20, 1953. Official Gaz. U. S. Pat. Office, **675**, 3: 736. 1953.

An insulated jacketed cabinet for cooling milk in cans. Circulation of the cooling medium is continued until a thermostat, located in the coolant leaving the jacket, operates to stop the flow. R. Whitaker

ICE CREAM

1073. Algin stabilizers in chocolate mixes. G. D. Sperry, Research Dept., Kelco Company, San Diego, Calif. Ice Cream Rev., **37**, 3: 74. 1953.

Results of comparative trials with four algin stabilizers (Dariloid, Dariloid XL, Dariloid K and Sherbilizer) on the viscosity of chocolate mixes are presented. Each was used to stabilize mixes prepared with 3% Dutch process cocoa, 3% American process cocoa, and 4% by weight of a blend of Dutch process cocoa and chocolate liquor.

The effect of 3 different emulsifiers and 2 salts on the viscosity of chocolate mixes when used in conjunction with each of the algin stabilizers also was determined.

The mix used throughout contained 10% butterfat, 9% serum solids, 18% sugar, 3-4% chocolate, and 0.12-0.20% stabilizer. All mixes were pasteurized at 160° F. for 30 min. and homogenized at 1500 lb. pressure using a single stage viscolizer.

Sherbilizer (0.20%) yielded mixes of uniformly low viscosity. Little or no increase in viscosity was observed as a result of aging. Dariloid XL (0.20%) also produced mixes of low viscosity immediately after processing. There was a tendency for the mixes stabilized with Dariloid XL to increase in viscosity during the aging process. In no case, however, was an excessively high viscosity encountered even after aging. The use of 0.12% Dariloid K resulted in higher viscosities than when either Sherbilizer or Dariloid XL was used. The highest viscosities were encountered in the mixes prepared with American process cocoa, followed in order by the blend of Dutch process cocoa and liquor chocolate, and the Dutch process cocoa. The viscosities of the mixes stabilized with Dariloid K were below the maximum acceptable value of 1000 cps. Dariloid (0.20%) could be used satisfactorily in mixes prepared with Dutch process cocoa, but resulted in excessive viscosities when used in conjunction with American cocoa. When used in the mix prepared with the blend of cocoa and liquor the viscosity approached the maximum acceptable level.

The addition of 0.20% sodium hexametaphosphate to a chocolate mix was somewhat more effective than a similar amount of sodium citrate in reducing the viscosity. Either sodium hexametaphosphate or sodium citrate was more effective in reducing the viscosity of chocolate ice cream mixes than any of the three emulsifiers studied. The greatest reduction in viscosity was observed when 0.05 per cent polyoxyethylene sorbitan monostearate was used. The use of 0.05 per cent polyoxyethylene stearate, and 0.10 per cent glycerol monostearate was less effective.

W. J. Caulfield

1074. A study of some stabilizers in relation to HTST pasteurization of the ice cream mix. J. P. Julien, Provincial Dairy School, St. Hyacinthe, Quebec, Canada. Ice Cream Trade J., **49**, 9: 44. 1953.

Three stabilizers, FR-38FP, gelatin and C.M.C., were used in combination with an emulsifier and calcium sulphate to stabilize ice cream mixes which were heated in a HTST pasteurizer.

The highest body and texture score was given to the ice cream stabilized with 0.3% guar seed gum in cobination with 0.1% emulsifier and 0.1% calcium sulphate. The melt down rate was reduced when this stabilizer was used with an emulsifier. W. H. Martin

1075. Operation of continuous freezers for quality and efficiency. R. A. WEIR. Can. Dairy and Ice Cream J., **32**, 5: 44-47. 1953.

The paper presents in detail the factors involved in the operation and maintenance of continuous freezers. H. Pyenson

1076. HTST pasteurization of ice cream mix. H. B. SHEFFIELD, Branch Engineer, Cherry-Burrell Corp., San Francisco, Calif. Ice Cream Rev., **37**, 3: 116-118. 1953.

Approximately 20 ice cream plants in the U. S. are now using the HTST method of pasteurizing ice cream mix. The results obtained indicate that pasteurization of ice cream mix at 175° F. for 25 seconds will speed up production, reduce labor, steam and water costs, produce ice cream with low bacterial counts, not impair the flavor quality of the resulting ice cream, and produce mix with good freezing properties.

A description of several installations is presented as well as a discussion of the procedures used in preparing chocolate mixes, French ice cream, and mixes involving the use of frozen cream. W. J. Caulfield

1077. Continuous freezers. G. O. WYMOND, Cherry-Burrell Corporation, Detroit, Mich. Milk Plant Monthly, **42**, 8: 29-30. 1953.

The continuous freezer is more than a production machine. Its operating efficiency must be measured in terms of quality as well as the gal. of ice cream produced per hr. The operator must know the range of quality control in his freezer, be able to recognize the quality he wants, and operate the freezer to get that quality. He must realize that conditions which affect operation will vary. The freezer cannot be operated by any standard formula. Gauge readings, capacity settings, and the temperature of the product as it is discharged from the freezer will vary. The best indicator of quality is the stiffness of the ice cream as it is discharged. Operating adjustments should be made, as required, to freeze to the stiffness desired. C. J. Babcock

1078. How to obtain maximum efficiency from a continuous freezer. G. O. WYMOND, Cherry-Burrell Corp., Detroit, Mich. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 81-84. 1953.

The maximum rate at which the freezer should be operated is that at which the desired stiffness and dryness of ice cream can be obtained. Improper refrigeration has caused the greater part of difficulties in freezer operation. Insufficient refrigeration is most commonly due to oil in the system, but may be due to too small a compressor, inadequate size of suction or liquid liner and liquid refrigerant starvation.

The oil used in the system should have a pour point of minus 25° F. and a flash point of over 300° F. It should drain freely from metal surfaces and be wax free. Mix entering the freezer should be at a temperature of from 32° F. to 40° F. In sharpening freezer blades the old cutting edge should not be filed or cut away so the blades will not have to wear in before full scraping can be expected. Various difficulties are caused by dashers which are out of alignment, worn pumps and pumps which are out of adjustment. J. Tobias

1079. Vitamin A fortification of vegetable fat frozen dessert. H. B. CONANT and C. L. WRENSHALL, Chas. Pfizer & Co., Inc., Brooklyn, N. Y. Ice Cream Trade J., **49**, 9: 24-26. 1953.

Fortification of "Mellorine" by the addition of vitamin A acetate at the rate of 10,000 USP U per gal. to the mix before pasteurization was carried out in pilot plant and commercial batches.

Tests showed a maximum loss of 9.2% during processing and storage at 0° F. for 9 wks. No objectionable flavors were noted in the frozen product. Synthetic vitamin A palmitate also was found to be satisfactory. W. H. Martin

1080. New vending device provides variety in ice cream novelty sales. ANON. Ice Cream Trade J., **49**, 9: 53. 1953.

A new vending device which will handle almost any novelty including ice cream sandwiches, packaged cones, boxed confections, twin pops and bars, has been placed on the market by the Vendo Co., Kansas City, Mo. W. H. Martin

1081. Stabilized fruits make better ice cream. ANON. Md. Agr. Expt. Sta., College Park. 65th Ann. Rept., p. 45. 1951-52.

Recent studies indicate that when stabilizers are added to strawberries, they have a more desirable appearance in ice cream and more juice is retained in the fruit. Less bleeding and better fruit distribution also results. CMC, pectin and algin derivatives were used. R. W. Hunt

1082. Calcium sulfate effective in producing ice cream for special handling. ANON. Md. Agr. Expt. Sta., College Park. 65th Ann. Rept., p. 45. 1951-52.

A dry, stiff, slow melting ice cream was produced when 0.12% calcium sulfate was used in conjunction with some of the common stabilizers. R. W. Hunt

1083. Sanitary cream dispenser. J. F. PEAL. U. S. Patent 2,655,289. 4 claims. Oct. 13, 1953. Official Gaz. U. S. Pat. Office, **675**, 2: 440. 1953.

An easily cleaned hand operated dispenser for delivering uniformly small portions of cream. R. Whitaker

1084. Emulsification of ice-cream mixtures.

N. Savinovskii. Kholodil'naya Tekh., **30**, 1: 37-40. 1953. (Chem. Abstr., **47**: 10146a. 1953.) S. Patton

1085. Bacterial nitrate reduction for the hygienical supervision of ice creams. R. Attimonelli, Univ. Bari, Italy. Igiene e sanità pubblica, **8**: 608-612. 1952. (Chem. Abstr., **47**: 8922a. 1953.) S. Patton

1086. The nitro reduction test in the hygienic supervision of ice creams. B. Scotti, Lab. batteriol. micrograf., Grosseto, Italy. Igiene e sanità pubblica, 9: 55-56. 1953. (Chem. Abstr., **47**: 8922b. 1953.) S. Patton.

MILK AND CREAM

1087. A study of the cell counts of milk from healthy cows. P. MacLeod and E. O. Anderson. Storrs Agr. Expt. Sta., Conn. Bull. 290. 1952.

Three studies on cell counts of milk from healthy cows, both individually and as a herd, are reported. The first study dealt with cell counts of milk from 10 healthy cows during the 2nd through the 41st wk. after parturition to provide a statistically tested estimate of the cell count of normal milk. The geometric mean cell count of 10 cows over the 40 wk. period was approximately 70,000. The second study was concerned with cell counts of 20 healthy cows from the 1st to the 21st day after parturition. The ave. cell count of the milk from this group of cows was 150,000, with a steady decline after the 1st wk. The third study was conducted over a 17 mo. period to determine whether a significant difference appeared in the cell count of A.M. and P.M. milk. A small but significant difference was found to exist between milkings, the P.M. count being slightly higher than the A.M. R. W. Hunt

1088. An evaluation of thermal effectiveness of the pasteurizing process in commercially operated HTST pasteurizers. J. L. Foley and D. J. Hankinson, Univ. of Mass., Amherst. Presented at meeting E. Div. A.D.S.A. Sept. 21, 1953.

Ten commercial installations of HTST milk pasteurizers were evaluated. Thermocouples were inserted between the plates in the heating section and pasteurized regenerator section to obtain temperature readings at selected points. Using flow rates and volume data for each unit, a time-temperature relationship was established. From these values and established destruction rates for the phosphatase enzyme, lethality curves were constructed.

The pasteurizers studied contributed from 213 to 390% of the heat necessary to inactivate the phosphatase enzyme. The heating and cooling portions of the pasteurization treatment contributed from 20 to 108% of the heat needed to inactivate the phosphatase enzyme. Operation of the pasteurizers above the legal requirements contributed far more to the total lethal heat than did the rate of heating and cooling.

Author's Abstract

1089. Keeping quality of unfrozen concentrated milk. D. Rose and I. J. McDonald. Can. Dairy and Ice Cream J., **32**, 2: 38. 1953.

Good quality whole milk will keep longer than concentrates of poor quality milk, but with equal initial contamination the concentrate should remain sweet about twice as long as whole milk. In 2 wks. a 3:1 concentrate at 40° F. had a sediment of lactose crystals which made it undesirable from a consumer standpoint. Such factors as savings in home refrigerator space and in bulk space indicate a potential market of 3:1 concentrate, but only careful study of local conditions can indicate whether the market is sufficiently large to make productions economically sound.

H. Pyenson

1090. Needed: 145 billion pounds of milk a year. Anon. Milk Products J., **44**, 10: 26-27. 1953.

This story describes the over-all research and education activities of the National Dairy Council. The Dairy Council program is dedicated to stimulating the increased consumption of dairy products. It has estimated that if 160 million consumers all observed the recommended daily allowances for good nutrition, 145 billion lbs. of milk per annum would be needed to meet such market demand.

Aspects such as research projects, utilization of research, financing and dividends on investment are discussed. J. J. Janzen

1091. Good buttermilk. M. W. Ransom, Dairy Laboratories, Philadelphia, Pa. Milk Plant Monthly, **42**, 8: 39-40. 1953.

In the preparation of buttermilk, the desired quantity of milk should be held at 190° F. for 20-30 min., cooled to 72° F. and 2 qts. of starter added for every 40 qts. of milk. It should be thoroughly agitated and held at a temperature as near 72° F. as possible until the milk becomes thick and attains an acidity of approximately 0.85%. The buttermilk should be agitated sufficiently to smooth the body and then cooled as promptly as possible to 50° F. using care not to incorporate air. Salt and butter granules may be added if desired. In plants manufacturing large amounts of fermented milks, a 1% inoculation of culture propagated daily gives satisfactory results. A 5% inoculation gives better results when the starter is propagated less frequently. C. J. Babcock

1092. Good cultures. N. C. Angevine, Meyer Blaneke Co., St. Louis, Mo. Milk Plant Monthly, **42**, 8: 60-62. 1953.

Good cultures have a firm, smooth body with

a velvety or creamy-like texture. They are free from all whey and signs of gas. The flavor and aroma is mild, pleasant, clean, and mildly acid. Titratable acidity should be about 0.80% to 0.85% at end of ripening period. Abnormal cultures usually are caused by either biological contamination or physical conditions. They are indicated by a gassy body, yeast or alcoholic flavor and aroma, no coagulation, lumpy or grainy curd, caramel flavor, ropiness, wheying off, or bitter flavors. C. J. Babcock

1093. Good flavored milk—A program for producer and processor. G. H. WILSTER and K. A. JARVIS. Ore. Agr. Expt. Sta., Corvallis. Circular 525. 1953.

The causes and prevention of common flavor defects in milk are reviewed. R. W. Hunt

1094. "Uperization," a new process for the treatment of milk. P. HAERRY, Konolfingen, Switz. Mitt. Lebensm. Hyg., **44**: 159-162. 1953. (Chem. Abstr., **47**: 8921f. 1953. S. Patton

1095. Apparatus for preparing foam-like substances such as whipped cream. L. B. HEESEN. U S. Patent 2,654,585. 3 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 198. 1953.

A vessel constructed to hold cream under gas pressure, a tube reaching to the bottom of the vessel to a double valve head, one valve being used to admit the gas to the chamber and the other for discharging the whipped product.

R. Whitaker

1096. Electric test bottle bath. G. E. SHAW (assignor of 1/2 to A. A. Morgan). U. S. Patent 2,653,214. 1 claim. Sept. 22, 1953. Official Gaz. U. S. Pat. Office, **674**, 4: 1056. 1953.

An electrically heated, thermostatically controlled, water bath suitable for tempering Babcock and other test bottles is described. The bottles are held in racks constructed of perforated metal and baffles are provided to insure uniform temperature throughout the bath.

R. Whitaker

1097. Cream dispensing apparatus for coffee-making and dispensing machines. E. J. LIME (assignor to Lymean Manufacturing Co., Inc.). U. S. Patent 2,652,176. 4 claims. Sept. 15, 1953. Official Gaz. U. S. Pat. Office, **674**, 3: 715. 1953.

A valve is described for dispensing dried cream in a coffee vending machine.

R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

1098. Effect of cooking on the vitamin A content of ghee (clarified butter). I. S. BHATIA, Central Food Technol. Research Inst., Mysore. Bull. Central Food Technol. Research Inst., Mysore, **2**: 72-74. 1952. (Chem. Abstr., **47**: 9517a. 1953.) S. Patton

1099. Skim-milk powders and experimental rat caries. F. J. MCCLURE and J. E. FOLK, Natl. Inst. Dental Research, Bethesda, Md. Proc. Soc. Exptl. Biol. Med., **83**: 21-26. 1953. (Chem. Abstr., **47**: 8849h. 1953.) S. Patton

1100. Treatment of kwashiorkor with milk and vegetable proteins. R. F. A. DEAN, Cambridge Univ., Engl. Brit. Med. J., II: 791-796. 1952. (Chem. Abstr., **47**: 10073f. 1953.)

B. L. Larson

1101. Heat processing and the nutritive value of milk and milk products. L. J. SCHROEDER, M. IACOBELLIS, and A. H. SMITH, Wayne Univ., Detroit, Mich. J. Nutrition, **49**: 549-561. 1953. (Chem. Abstr., **47**: 10073i. 1953.)

B. L. Larson

1102. Effect of vitamin B_{12} in the diet of the rat on the vitamin B_{12} contents of milk and livers of young. L. J. DANIEL, M. GARDINER, and L. J. OTTEY, Cornell Univ., Ithaca, N. Y. J. Nutrition, **50**: 275-289. 1953. (Chem. Abstr., **47**: 10075i. 1953.) B. L. Larson

1103. Feeding infants with condensed milk as compared with usual milk mixtures. W. DROESE and H. STOLLEY, Univ. Kiel, Ger. Monatsschr. Kinderheilk., **101**: 285-292. 1953. (Chem. Abstr., **47**: 10079h. 1953.)

B. L. Larson

1104. Vitamin B_{12} and folic acid in milk diets. R. A. COLLINS, M. SCHREIBER, C. A. ELVEHJEM, and E. B. HART, Univ. Wisc., Madison. J. Nutrition, **49**: 485-494. 1953. (Chem. Abstr., **47**: 9440h. 1953.) B. L. Larson

PHYSIOLOGY AND ENDOCRINOLOGY

1105. Respiratory activity as an index of heat tolerance in Jersey and Sindhi X Jersey (F_1) Crossbred cows. R. E. MCDOWELL, D. H. K. LEE, M. H. FOHRMAN, and R. S. ANDERSON. J. Animal Sci., **12**, 3: 573-581. 1953.

Jersey cows and Red Sindhi × Jersey (F_1) cows were observed at a normal temperature of 57-84° F., and again after 3 hrs. exposure to 105° F. with a vapor presure of 34 mm Hg. Rectal temperature, respiratory rate, and respiratory volume were measured. The crossbred cattle showed a smaller rise in rectal temperature (1.87 ± 0.152° F.) than did the Jerseys (3.06 ± 0.236° F.) The correlation between respiratory volume and rectal temperatures in cows exposed to comparable heat stress was positive and often of high magnitude. Respiratory rate proved to be a less sensitive index of heat tolerance than respiratory volume. Under the conditions of the experiment there

was no significant relationship between rectal temperature and respiratory rate.
O. T. Stallcup

1106. Replacement therapy with a slowly absorbed progesterone product. L. E. McDonald, R. E. Nichols, and S. H. McNutt, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **14**, 52: 385-387. 1953.

Five cows were used to study the efficiency of an aqueous solution of microcrystalline progesterone in maintenance of pregnancy. After extirpation of the corpus luteum at 63 to 94 d. of gestation, therapy was started at the rate of 500 mg. progesterone every 10 d. in 3 cows, and every 13 d. in 2 cows. Dosage was discontinued at 143 to 174 d. gestation in 3 cows and pregnancy was maintained. One cow aborted at 120 d. after dosage stopped on the 103rd d. One of the 13-d. dosed cows resorbed the fetus.
E. W. Swanson

1107. The nerve control of rumination and reticulo-ruminal motility. C. H. Clark, Coll. of Vet. Med., Ohio State Univ., Columbus. Am. J. Vet. Research. **14**, 52: 376-384. 1953.

Sheep were used to study the effects of altering nerve and brain centers upon the activity of the rumen. Cutting the vagus nerves stopped reticulum activity while stimulation of the vagi caused reticulum contraction. This confirms that the vagi are the motor nerves to the rumen and the reticulum. Removal of the cerebral cortex did not prevent rumination or rumen motility. Complete removal of the cerebrum resulted in no rumen motility. Frontal lobectomy did not destroy the ability to ruminate, but caused abnormally long ruminating periods. A center for rumination is postulated as being in a subcortical area anterior to the pituitary infundibulum.
E. W. Swanson

1108. Extremely high alkaline phosphatase activity in the vaginal mucus of the cow. G. A. Van Klinkenberg. Nature, **172**, 4374: 397. 1953.

The vaginal mucus of cows is extremely high in phosphatase activity 1-2 d. pre-oestrum. The enzyme can be prepared from the mucus in dry stable form, with high potency, by pption. with cold ethanol or acetone.
R. Whitaker

1109. The apparent digestibility of nitrogen as associated with calcium fertilization of the soil. K. A. Kendall, R. W. Touchberry, W. B. Nevens, and O. R. Overman. J. Animal Sci., **12**, 3: 635-641. 1953.

Using the rabbit as an experimental animal the authors observed that the apparent digestibility of the N in lespedeza hay and in wheat grain was significantly higher when these crops were grown under a Ca-P-K fertilizer system than when the crops were grown on adjoining plots given a P-K fertilizer treatment. Early harvested lespedeza hay had a significantly higher coefficient of apparent digestibility of N than did late harvested hay.
O. T. Stallcup

1110. Influence of a high salt intake on cellulose digestion. B. P. Cardon. J. Animal Sci., **12**, 3: 536-540. 1953.

Digestion studies with Hereford cows and artificial rumen fermentation experiments indicate that an increased salt concentration in the rumen caused by feeding high salt rations did not decrease rumen microbial activity. Both types of experiments indicate that digestible cellulose as well as digestible gross energy are not altered by the increased consumption of salt.
O. T. Stallcup

1111. Chemical changes in milk fat related to flavor in dairy products. Anon. Md. Agr. Expt. Sta., College Park. 65th Ann. Rept., p. 43. 1951-52.

A study was made of milk given during both natural and induced estrus. The free fatty acids and lipase activity increased during the estrum period, with the highest point in lipase activity being reached the day of estrus and the peak of fatty acids occurring the following day.
R. W. Hunt

1112. Utilization of dietary carbohydrates by ruminants. I. R. Jarrige, Inst. natl. agron., Paris. Ann. nutrition et aliment., **7**: 245-302. 1953. (Chem. Abstr., **47**: 8847e. 1953.)
S. Patton

1113. Mineral metabolism in growing calves. I. A. Tremazi, Agr. Coll & Research Inst., Lyallpur, Pakistan. Pakistan J. Sci. Research, **4**: 45-50. 1952. (Chem. Abstr., **47**: 8850b. 1953.)
S. Patton

1114. Biochemical studies on vitamin B_{12}. III. Vitamin B_{12} deficiency in cattle and biosynthesis of the vitamin in vivo. Y. Sahashi and K. Iwamoto, Univ. Tokyo. Proc. Japan Acad., **28**: 573-577. 1952. (Chem. Abstr., **47**: 8850g. 1953.)
S. Patton

1115. Vitamin B_{12} content of calf thymus extract. W. J. Kaipainen, A. Telkkä, and K. K. Mustakallio, Univ. Helsinki, Fin. Nature, **171**: 845. 1953. (Chem. Abstr., **47**: 8862a. 1953.)
S. Patton.

1116. Action of iodoproteins on milk secretion. F. Usuelli and G. Piana. Boll. soc. ital. biol. sper., **26**: 1439-1442. 1950. (Chem. Abstr., **47**: 8865b. 1953.)
S. Patton

1117. The plasmalogen content of sperm. W. Boguth, Justus Liebig Hochschule, Giessen, Ger. Naturwissenschaften, **39**: 432. 1952. (Chem. Abstr., **47**: 8870b. 1953.)
S. Patton

1118. Reducing substances in the cervical mucus of the cow. K. KAEMMERER and H. G. NEUMANN, Univ. Bonn, Ger. Z. Tierzücht. Züchtungsbiol., **58**: 416-421. 1950. (Chem. Abstr., **47**: 8874f. 1953.) S. Patton

1119. Lactation in virgin rabbits after treatment with simple substances without hormone administration. I. GY. FAZEKAS, Univ. Szeged, Hung. Endokrinologie, **30**: 45-54. 1953. (Chem. Abstr., **47**: 8875i. 1953.) S. Patton

1120. The utilization of milk by young animals. A. M. LEROY, G. LERY, and S. ZELTER, Natl. agron. inst., Paris. Ann. inst. natl. recherche agron., Sér. D. Ann. zootech., **1**, 1: 61-77. 1952. (Chem. Abstr., **47**: 8864a. 1953.) S. Patton

1121. Adenosinetriphosphatase effect of uterine myosin. A. WESTGREN. Acta Endoerinol., **5**: 425-432. 1950. (Chem. Abstr., **47**: 10017b. 1953.) B. L. Larson

1122. Spermine oxidase: An amine oxidase with specificity for spermine and spermidine. J. G. HIRSCH, Rockefeller Inst. Med. Research, New York. J. Exptl. Med., **97**: 345-355. 1953. (Chem. Abstr., **47**: 10023e. 1953.) B. L. Larson

1123. Determination of glucose in the semen. A. TRABUCCO and R. ENCINA. Obstet. y ginecol. latinoamer., **10**: 508. 1952. (Chem. Abstr., **47**: 10041a. 1953.) B. L. Larson

1124. The carotenoid composition of the blood, liver, and ovaries of the rat, ewe, cow, and frog. J. GANGULY, J. W. MEHL, and H. J. DEUEL, JR., Univ. of S. Calif., Los Angeles. J. Nutrition, **50**: 73-83. 1953. (Chem. Abstr., **47**: 10076i. 1953.) B. L. Larson

1125. Bovine erythrocyte antigens. III. The isolation of blood-group specific substances from erythrocytes. G. C. ROYAL, L. C. FERGUSON, and T. S. SUTTON, Ohio State Univ., Columbus. J. Immunol., **71**: 22-25. 1953. (Chem. Abstr., **47**: 10092e. 1953.) B. L. Larson

1126. Serum proteins in tuberculous cows. J. P. MUZZO, Univ. nacl. mayor San Marcos, Lima, Peru. Rev. fac. med. vet. Univ. nacl. mayor San Marcos, Lima, Peru, **6**: 184-189. 1951. (Pub. 1952). (Chem. Abstr., **47**: 10092i. 1953.) B. L. Larson

1127. The toxity of heated serum albumin. P. FALISE, Univ. Louvain, Belg. Arch. intern. pharmacodynamie, **92**: 228-240. 1952. (Chem. Abstr. **47**: 10103f. 1953.) B. L. Larson

1128. Growth stimulation and growth inhibition of chicks fed forage and forage-juice concentrate. R. G. HANSEN, H. M. SCOTT, B. L. LARSON, T. S. NELSON, and J. KRICHEVSKY, Ill. Agr. Expt. Sta., Urbana. J. Nutrition, **49**: 453-464. 1953. (Chem. Abstr., **47**: 9440c. 1953.) B. L. Larson

1129. The effect of large doses of estrogens on the metabolism and fattening of cattle. W. KOCH, Univ. Munich, Ger. Berlin. u Münch. tierärztl. Wochschr., 211-213. 1950. Chem. Zentr., **1**: 1474. 1951. (Chem. Abstr., **47**: 9446h. 1953.) B. L. Larson

1130. Biochemical studies on vitamin B_{12}. VI. Relation of vitamin B_1 and vitamin B_{12} to energy metabolism in cattle. Y. SAHASHI and K. IWAMOTO, Tokyo Univ., Proc. Japan Acad., **29**: 33-35. 1953. (Chem. Abstr. **47**: 9446b. 1953.) B. L. Larson

1131. Observations on the feeding of the mineral feed supplement animalin with vitamin D. to young cattle. G. SCHLETTER. ZÜCHTUNGSKUNDE, **22**: 28-32. 1950. Chem. Zentr., **1**: 77-78. 1951. (Chem. Abstr., **47**: 9447e. 1953.) B. L. Larson

1132. Carbonate and fatty acids as precursors of amino acids in casein. A. L. BLACK, M. KLEIBER, and A. H. SMITH, Univ. Calif., Davis. J. Biol. Chem., **197**: 365-370. 1952. (Chem. Abstr., **47**: 9469h. 1953.) B. L. Larson

1133. Acetate as a precursor of milk constituents in the intact cow. M. KLEIBER, A. H. SMITH, A. L. BLACK, M. A. BROWN, and B. M. TOLBERT, Univ. Calif., Davis. J. Biol. Chem., **197**: 371-379. 1952. J. Animal Sci., **11**: 61. 1952. (Chem. Abstr., **47**: 9469i. 1953.) B. L. Larson

1134. Carbonate as precursor of milk constituents in the intact dairy cow. M. KLEIBER, A. H. SMITH, and A. L. BLACK, Univ. Calif., Davis. J. Biol. Chem., **195**: 707-714. 1952. (Chem. Abstr., **47**: 9470c. 1953) B. L. Larson

1135. Action of veratrine and emetine on the gastric motility of ruminants. M. BRUNAUD and M. DUSSARDIER, École natl. vét., Toulouse, France. J. Physiol. (Paris), **44**: 224-228. 1952. (Chem. Abstr., **47**: 9492a. 1953.) B. L. Larson

SANITATION AND CLEANSING

1136. Progress in sanitation chemistry. R. B. BARRETT, Klenzade Products Inc., Beloit, Wis. Sou. Dairy Prod. J., **54**, 3: 120. 1953.

Synthetic detergents, chelating agents, QAC, polyphosphates and C.I.P. cleaning techniques are cited as recent important accomplishments of applied research in sanitation chemistry. Further progress is dependent upon new ave-

nues which may be created by pure research. Progress has been made in laboratory detergency evaluation but performance testing is still the best test of a product or method. Chelates are especially valuable as sequestering agents, for thermal stability, inactivation of certain microorganisms under some conditions, decontamination of surfaces of radioactive materials and in cleaning operations involving alternate usage of alkalies and acids. The increased use of wetting agents has greatly increased cleaning efficiency and has caused a downward trend in soap consumption. Fluorocarbons, although too expensive, have the advantage of reducing the surface tension of solutions to only about ½ that of commercial wetting agents. C.I.P. operations are being adapted to more and more types of equipment.

F. W. Bennett

1137. Sanitation bacteriology. P. R. ELLIKER, Dept. of Bacteriol., Ore. State Coll., Corvallis. Milk Plant Monthly, **42**, 8: 31-32. 1953.

One of the most important advances in sanitation bacteriology has been the development of inexpensive and highly effective chemical sanitizing agents and germicides. There are several types, but hypochlorites and quaternary ammonium compounds are most widely used. The pH of the germicidal solution is important in that activity increases as the pH decreases. This information has enabled the preparation of commercial hypochlorites adjusted to pH levels that provide stability and a high rate of germicidal activity. Research on the effect of organic material on hypochlorite stability has emphasized that no utensil or equipment surface can be treated effectively with a chemical sanitizer unless that surface is clean. This requirement equally applies to QAC and other chemical germicides. The degree that fundamental research on cleaners and sanitizers has been applied to advantage in recent years is illustrated by specific problems in the control of coliform, thermoduric, and thermophilic, and psychrophilic bacteria, and bacteriophage.

C. J. Babcock

1138. Recirculation methods for cleaning dairy pipe lines. R. F. HOLLAND, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 12-14. 1953.

Permanent pipe lines have been constructed of stainless steel, glass and plastics. The latter have not proven satisfactory. It is important that unions be rigid and equipped with non-absorbent, durable gaskets producing flush joints. Whenever a joint is opened the gasket should be replaced with a new one. Dead ends longer than 1½ times of the pipe diameter must be avoided. A flow rate of 2.5 ft. per sec. or higher was found satisfactory for lines transporting chocolate milk and pasteurized milk. Directions are given for the treatment of cold and hot milk lines.

J. Tobias

1139. Correctly designed cleaning equipment for dairy plants. J. R. PERRY, National Dairy Prod. Co., Inc., New York, N. Y. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 15-28. 1953.

Properly tempered rinse water is essential for good cleaning. A temperature of 115° F. was found most satisfactory. The best and most economical way to obtain such water is by means of a hot water generator and a tempering valve.

A controlled pressure water station either of the open or cabinet type form will satisfactorily control the pressure of the rinse water. It will be possible to use a light-weight hose with an inside diameter of ½ in. Economies also will be realized if a shut-off valve is connected to the smaller hose along with a whip-end, which will not kink or crack. The shut-off valve may be equipped with either a blade or round nozzle depending on the type of rinsing to be done.

A sanitary pipe cleaner was developed which in one operation scrubs both the inside and outside of the pipe, and in a second operation rinses both sides with tempered water which is only used once. Special outside brush assembly and rinsing assembly have been developed which may be installed on existing pipe washing equipment. A sanitary pipe truck whose frame, hooks, and shelves are coated with neoprene, should be used to transport pipes to and from the pipe cleaner.

Sanitary fittings are washed best by either a sanitary fittings cleaner or combination parts cleaner. The latter was designed to permit the washing and rinsing of separator and clarifier dises in addition to small parts and fittings. Parts to be washed should be brought to the cleaning unit on a sanitary fitting buggy.

For scrubbing processing equipment and other surfaces a combination of cleaning solution tank, cleaning solution hose, small shut-off valves and solution fed brushes should be used.

A portable cleaner table was developed to fill the cleaning needs of small plants and small departments.

Other new developments include a special light for cleaning, chlorine spray sterilizer, sanitary fittings wrench with hammer members, a new coating material for certain of the new cleaning aids and a spray cleaning assembly for product storage tanks and milk truck tanks.

In practicing "in line cleaning" it was found advantageous to use a portable cleaning solution tank assembly where several circuits were to be cleaned and to utilize rinse water and cleaning solution whose temperature is 115° F. when it leaves the circuits.

J. Tobias

1140. Progress in sanitary standards for dairy equipment. E. H. PARFITT, Sanitary

Standards Subcommittee of the Dairy Industry Committee. Proc. First Ann. Dairy Eng. Conference, Mich. State Coll., East Lansing. p. 29-36. 1953.

A review of the participation of the Dairy Industry Committee in the 3A Sanitary Standards Committee is presented. A number of basic points established in one or more of the sanitary standards are enumerated.

J. Tobias

1141. Studie over enkele eigenschappen van reinigingsmiddelen (Study on some properties of detergents). English summary. A. De Vleeschauwer, E. Wallez, H. Hendrickx, and M. Naudts, Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent., **17**, 4: 566-596. 1952.

Titration curves of singular and composite detergents were determined. The removal of milk films was studied with a great number of detergents at different concentrations and temperatures. W. C. van der Zant

1142. Dairy plant brushes. J. Larson, Sparta Brush Co., Sparta, Wis. Milk Dealer **42**, 11: 40-41. 1953.

Brush construction may be classified as staple set, wire drawn, twisted in wire, rubber, plastic, or pitch set, and spiral wound. Filling materials may be classified into vegetable fibres processed from plants, animal hair obtained principally from wild hogs and horses, synthetic fibres such as nylon and plastic and wire such as nickel, silver and stainless steel.

To obtain greater brush life, they should be thoroughly cleaned after use and hung up to dry. This also prevents odor absorption especially by vegetable fibre brushes. Bacterial contamination can exist in any type of brush, either on the fibre or in it, if the brush is not cleaned and sanitized. C. J. Babcock

1143. Automatic sanitary apparatus for dispensing measured quantities of liquid. R. E. Corradini, L. S. Lynch, O. Bonomi, and G. C. Engel (assignors to Robert E. Corradini). U. S. Patent 2,654,517. 36 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 177. 1953.

An automatic dispenser which draws milk by vacuum from a conventional milk can and discharges a uniform quantity into a drinking cup, independent of the amount of milk in the can. R. Whitaker

1144. Power washing cream separator. H. A. Heckendorf (assignor to International Harvester Co.). U. S. Patent 2,654,536. 10 claims. Oct. 6, 1953. Official Gaz. U. S. Pat. Office, **675**, 1: 183. 1953.

Details are given for the design of a separator bowl which can be cleaned by a solution without disassembling the bowl. The outer side walls are shaped so that the sediment collects in a horizontal V-shaped groove. The bottom of the groove is sealed by a soft rubber ring when the bowl is operating at the normal separating speed, but which bends to unseal the bowl at slow speed and allows the washing solution to escape into an outer shell for discharge outside the bowl housing. R. Whitaker

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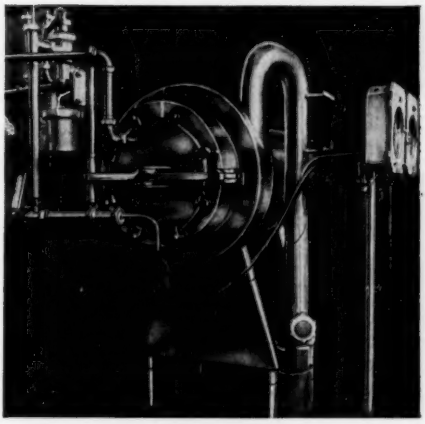
Mojonnier 84-5 Tubular Heater in Philadelphia Dairy Products Company's Athens St. plant, Ardmore, Pa. This unit has special highly polished stainless steel finish and meets the sanitary standards of Ardmore County, considered among the highest in the world.



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● The new Mojonnier Tubular Heater is only 5 feet long and has 84 one inch tubes. It is operated at 6,000 pounds of raw milk per hour heated from 38° to 145° F. and has a maximum capacity of 8,000 pounds per hour. The Mojonnier Heater corrected unfavorable working conditions created by the old heater, which leaked steam and hot water. Cleaning, which can be accomplished by recirculation of acid and alkali solutions, is much easier with the new unit.

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